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METAL EXPOSURES, ENDOCRINE FACTORS AND CANCER RISK

DOCTORAL THESIS

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METAL EXPOSURES, ENDOCRINE FACTORS AND CANCER RISK

Exposición a metales, factores endocrinos y riesgo de cáncer

Esther García García-Esquinas

To my parents.

Dr. Marina Pollán Santamaría and Dr. Ana Navas Acién, inform that the thesis entitled *"Metal exposures, endocrine factors and cancer risk"* is an original work carried out by Esther García García-Esquinas under our guidance and supervision. This is an original work and has not been submitted to any university for the awarding of any degree/diploma. We verify that we have read the thesis, that it is well written and it demonstrates a thorough understanding of the scientific methodology.

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Preface

The present dissertation entitled “Metal exposures, endocrine factors and cancer risk” is the result of research performed in the last 3 years under the leadership of Esther García García-Esquinas in collaboration with members of the Departments of Environmental Health Science (Johns Hopkins Bloomberg School of Public Health), and Environmental and Cancer Epidemiology (National Center for Epidemiology, Institute of Health Carlos III).

The dissertation is organized into 6 chapters as follows. Chapter 1 provides a general introduction. Chapter 2 describes the main hypothesis and objectives. Chapter 3 gives an overview of the methods and study populations. Chapter 4 presents the main results: first, it evaluates the association of arsenic exposure and cancer mortality using data from a prospective cohort study in American Indians (Strong Heart Study); second, it investigates the association of cadmium exposure and cancer mortality in the Strong Heart Study; third it evaluates the possible effect of cadmium as a mediator on the association between cigarette smoking and cancer mortality; fourth it evaluates the association between several anthropometrical factors during lifetime and the risk of hormone-dependent tumors in a case-control study (MCC-Spain); and fifth it assesses the influence of diabetes, diabetes duration and diabetes treatment on the incidence of postmenopausal breast and prostate cancer in MCC-Spain. Chapter 5 provides a summary and discussion of the research findings, and chapter 6 completes the dissertation by presenting the main conclusions.

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LIST OF ABBREVIATIONS

AI: American Indians

BC: Breast Cancer

BMI: Body Mass Index.

CI: Confidence Interval

DMA: Dimethylarsenate

ER: Estrogen Receptor

HR: Hormone Receptor

IARC: International Agency for Research on Cancer

IC: Intervalo de confianza

ICD-9: International Classification of Diseases, 9th Revision

IQR: Interquartile range

MMA: Monomethylarsenate

NHANES: National Health and Nutrition Examination Survey

PC: Prostate Cancer

PR: Progesterone Receptor

SHS: Strong Heart Study

TN: Triple Negative

WC: Waist Circumference

WHR: Waist to Hip Ratio

SUMMARY

Cancer is one of the leading causes of death both in men and women in the world, particularly in developed countries. Around 90% of cancers are linked to some type of environmental or lifestyle factor, including use of tobacco, consumption of certain foods or exposure to substances in air, water and soil. Cancer epidemiology studies have played an important role in identifying many of these environmental factors and translating this knowledge into prevention strategies. However, in spite of extensive investigations, some gaps in the state of knowledge exist, and for some cancers like prostate cancer, few environmental factors have been identified. The main objective of this thesis is to address the influence of some of these environmental and lifestyle factors on cancer development.

Data from a cohort study (Strong Heart Study) and a case-control study (MCC-Spain) have been analyzed. The Strong Heart Study is the largest epidemiologic study of cardiovascular disease in American Indian populations ever undertaken. Initially conducted in 1989 to 1991, the Strong Heart Study recruited 4,549 participants from 13 tribes and communities in Arizona, Oklahoma, North and South Dakota and followed them up through 2008. MCC-Spain is a population-based multicase-control study that recruited incident, histologically confirmed cases of breast (N=1743) and prostate (N=1113) cancers from 22 Spanish public hospitals between 2008 and 2013. Controls (N=1880 for breast and 1460 for prostate) were frequency-matched to the cases, taking into account age, sex and region. This thesis first evaluates the association between low-to-moderate levels of exposure to certain metals (arsenic and cadmium) and cancer mortality in the Strong Heart Study population, using Cox proportional hazards models. Then, it investigates the role of diabetes and obesity in the development of hormone-dependent tumors (breast and prostate) in MCC-Spain, using logistic mixed models that included the interviewer or the study region as random effect terms.

The median (interquartile range) urine concentration for inorganic plus methylated arsenic species was 9.7 (5.8-15.9) µg/g creatinine. The adjusted hazard ratios (95%CI) comparing the 80^{th} versus 20^{th} percentiles of arsenic were 1.14 (0.92-1.41) for overall cancer, 1.56 (1.02-2.39) for lung cancer, 1.34 (0.66-2.72) for liver cancer, 3.30 (1.28-8.48) for prostate cancer and 0.44 (0.14-0.96) for lymphatic and hematopoietic cancers. The median (interquartile range) urine cadmium concentration was 0.93 (0.61-1.46) µg/g creatinine. The adjusted hazard ratios (95%CI) comparing the 80^{th} versus 20^{th} percentiles of cadmium were 1.30 (1.09-1.55) for total cancer mortality, 2.27 (1.58-3.27) for lung cancer and 2.40 (1.24-1.96) for pancreas cancer mortality.

In postmenopausal women, the age at maximum height was inversely associated with the risk of breast cancer ($OR_{\text{per year}}$:0.93; 95%CI:0.89-0.98), while an overall increased risk of this tumor was observed with increasing body mass index ($OR_{2\text{ units}}$:1.10; 95%CI:1.05-1.15), waist circumference ($OR_{10\text{ cm}}$: 1.04; 95%CI:0.99-1.20) and waist to hip ratio ($OR_{0.10\text{ units}}$:1.20; 95%CI:1.03-1.41). In premenopausal women, the weight at age 45 ($OR_{5\text{ kgs}}$:1.13; 95%CI:1.00-1.28) and the waist circumference were associated with breast cancer risk ($OR_{10\text{ cm waist circumference}}$:1.18; 95%CI:0.98-1.42) after adjustment for body mass index. By intrinsic subtypes, in postmenopausal women weight gain since age 20 was associated with an increased risk of HR+/HER2- tumors in models that did not account for body mass index ($OR_{5\text{ kg}}$:1.10; 95%CI:1.04-1.16), while displayed a negative association with HER2+ tumors (p heterogeneity of effects= 0.05). Those variables associated to obesity earlier in like ("weight at age 20" and "age at maximum weight) were associated with a decreased risk of more aggressive tumor subtypes (HER2+ and triple negative) in premenopausal but not postmenopausal women. In men, the age at maximum weight ($OR_{5\text{ years}}$: 1.05; 95%CI: 1.00-1.10) and the waist to hip ratio ($OR_{0.1\text{ units}}$:1.21; 95%CI:1.03-1.43) were associated with the risk of high-grade prostate tumors.

Self-reported diabetes was associated with an increased risk of triple negative breast tumors in postmenopausal women (OR:2.13; 95%CI:1.25-3.63). In this group of women, metformin use was associated with a decreased risk of HR+/HER2- tumors ($OR_{\text{per year}}$:0.89; 95%CI:0.80-0.99), while use of sulfonylurea was associated with an increased risk of triple negative tumors ($OR_{\text{per year}}$:1.10; 95%CI:1.00-1.20). Finally, an increased risk of postmenopausal breast cancer was observed in women under insulin treatment (OR:2.14; 95%CI:1.13-4.09), with a non-significant positive dose response association between years of insulin use and breast cancer risk ($OR_{\text{per year}}$:1.10; 95%CI:0.98-1.23). For prostate cancer, a decreased risk of low-grade tumors was observed in men with diabetes (OR:0.70; 95%CI:0.46-0.90), and this protective effect was stronger as time since diagnosis increased ($OR_{\text{per year}}$:0.94; 95%CI:0.87-1.00).

These results provide novel evidence about the influence of low-moderate arsenic and cadmium exposure levels on cancer mortality. Additionally, regarding the association of obesity and diabetes with hormone-dependent cancers (breast and prostate), they offer valuable information suggesting differences in the effect of these factors by tumor subtype.

RESUMEN

El cáncer es una de las principales causas de muerte en el mundo, particularmente en países desarrollados. Alrededor del 90% de los cánceres están relacionados con factores ambientales o estilos de vida, como por ejemplo la exposición a tabaco, el consumo de ciertos alimentos o la exposición a substancias tóxicas a través del aire, el agua o el suelo. Los estudios epidemiológicos ponen de manifiesto la importancia de los factores ambientales en el desarrollo del cáncer, facilitando el diseño de estrategias preventivas. A pesar de la cantidad de investigación existente hasta la fecha, hay todavía lagunas en el conocimiento, y para algunos tumores como el de próstata, se desconocen muchos de los agentes ambientales implicados. El objetivo de esta tesis doctoral es conocer la influencia de algunos de estos factores ambientales o estilos de vida en el desarrollo del cáncer.

Se han analizado datos de un estudio de cohorte (Strong Heart Study) y de un estudio de casos y controles (MCC-Spain). El Strong Heart Study es el estudio epidemiológico más grande sobre enfermedad cardiovascular realizado hasta la fecha en indios americanos. Durante los años 1989 y 1991 se reclutaron 4,549 participantes de 13 tribus y comunidades de Arizona, Oklahoma, Dakota de Norte y Dakota del sur, a los que se siguió hasta el año 2008. MCC-Spain es un estudio multicaso-control de base poblacional que reclutó durante los años 2008-2013, casos incidentes e histológicamente confirmados de cánceres de mama (N=1743) y próstata (N=1113) en 22 hospitales públicos españoles. Los controles (N=1880 para mama y 1460 para próstata) fueron apareados por frecuencia con los casos según grupos de edad, sexo y nodo de reclutamiento. Esta tesis evalúa en primer lugar la asociación entre la exposición a dosis bajas-moderadas de algunos metales (arsénico y cadmio) y la mortalidad por cáncer en el estudio Strong Heart Study, utilizando para ello modelos de riesgos proporcionales de Cox. Posteriormente, investiga el papel de algunos factores endocrinos (obesidad y diabetes) en el desarrollo de tumores hormono-dependientes (mama y próstata) en participantes del estudio MCC-Spain, utilizando para ello modelos mixtos de regresión logística que toman como efecto de términos aleatorios al entrevistador o al nodo reclutador.

La mediana (rango intercuartílico) de arsénico inorgánico y sus especies metiladas en orina fue de 9.7 (5.8-15.9) µg/g creatinina. Las hazard ratios (IC95%) comparando los percentiles 80 y 20 de la distribución de arsénico fueron 1.14 (0.92-1.41) para mortalidad global por cáncer, 1.56 (1.02-2.39) para mortalidad por cáncer de pulmón, 1.34 (0.66-2.72) para mortalidad por cáncer de hígado, 3.30 (1.28-8.48) para mortalidad por cáncer de próstata y 0.44 (0.14-0.96) para mortalidad por tumores del sistema linfohematopoyético.

La mediana (rango intercuartílico) de cadmio en orina fue de 0.93 (0.61-1.46) µg/g creatinina. Las hazard ratios (IC95%) comparando los percentiles 80 y 20 de la distribución de cadmio fueron 1.30 (1.09-1.55) para mortalidad global por cáncer, 2.27 (1.58-3.27) para cáncer de pulmón y 2.40 (1.24-1.96) para mortalidad por cáncer de páncreas.

En mujeres postmenopáusicas, la edad de máxima altura se relacionó inversamente con el riesgo de padecer cáncer de mama ($OR_{año}$:0.93; 95%IC:0.89-0.98), mientras que se observó un incremento del riesgo de padecer este tumor en mujeres con un mayor índice de masa corporal ($OR_{2\ unidades}$:1.10; 95%CI:1.05-1.15), una mayor circunferencia de cintura ($OR_{10\ cm}$:1.04; 95%CI:0.99-1.20) o un mayor índice de cintura-cadera ($OR_{0.10\ unidades}$:1.20; 95%CI:1.03-1.41). En mujeres premenopáusicas, el peso a los 45 años ($OR_{5\ kgs}$:1.13; 95%CI:1.00-1.28) y el tamaño de la circunferencia de la cintura se asociaron con un incremento del riesgo de cáncer de mama ($OR_{10\ cm\ cintura}$:1.18; 95%CI:0.98-1.42). Por subtipos tumorales, en mujeres postmenopáusicas la ganancia de peso desde los 20 años se asoció con un incremento del riesgo de padecer tumores RH+/HER2- en aquellos modelos que no tenían en cuenta el índice de masa corporal ($OR_{5\ kg}$:1.10; 95%CI:1.04-1.16), y con un decremento del riesgo de padecer tumores HER2+ (p heterogeneidad de efectos= 0.05). Las variables relacionadas con la obesidad a edades más tempranas de la vida ("peso a los 20 años" y "edad de alcance del peso máximo") se asociaron con un decremento del riesgo de algunos subtipos tumorales (HER2+ y triples negativos) en mujeres premenopáusicas, pero no en mujeres postmenopáusicas. En los varones, la edad a la que se alcanzó el peso máximo ($OR_{5\ años}$:1.05; 95%CI: 1.00-1.10) y el índice de cintura cadera ($OR_{0.1\ unidades}$:1.21; 95%CI:1.03-1.43) se asociaron con el riesgo de padecer tumores de próstata de alto grado.

La diabetes auto-reportada se asoció con un incremento del riesgo de padecer tumores triple negativos en mujeres postmenopáusicas (OR:2.13; 95%CI:1.25-3.63). En este grupo de mujeres, el uso de metformina se asoció con un menor riesgo de desarrollar tumores RH+/HER2- ($OR_{por\ año}$:0.89; 95%CI:0.80-0.99), mientras que el tratamiento con sulfonilureas se relacionó con un incremento del riesgo de padecer tumores triple negativos ($OR_{por\ año}$:1.10; 95%CI:1.00-1.20). Por último, se observó un incremento del riesgo de cáncer de mama en mujeres postmenopáusicas que utilizaron insulina (OR:2.14; 95%CI:1.13-4.09), siendo también positiva (aunque no significativa) la asociación con el número de años en tratamiento con este fármaco ($OR_{por\ año}$:1.10; 95%CI:0.98-1.23). Los varones diabéticos tuvieron un menor riesgo de padecer tumores de próstata de bajo grado

(OR:0.70; 95%CI:0.46-0.90), y este efecto protector era más marcado con el paso del tiempo desde el diagnóstico inicial de diabetes ($OR_{por\ año}$:0.94; 95%CI:0.87-1.00).

Estos resultados aportan nueva evidencia sobre la influencia de la exposición a niveles bajos-moderados de arsénico y cadmio en la mortalidad por cáncer. Además, en relación a la asociación entre obesidad y diabetes con los tumores hormono-dependientes (mama y próstata), proporcionan información valiosa que sugiere un efecto diferencial de estos factores por subtipo tumoral.

1 INTRODUCTION

1.1 Cancer Epidemiology

The art of epidemiological thinking is to draw conclusions from imperfect data

-George W. Comstock –
(1915-2007)

1.1.1 The burden of cancer

1.1.1.1 A look at the global burden of cancer

Cancer is one of the leading causes of death both in men and women in the world, particularly in developing countries (1). The most common cancers worldwide are lung, breast, large intestine (colon and rectum), stomach and prostate, with lung cancer accounting for most deaths. Based on the GLOBOCAN 2008 estimates, around 12.7 million new cancer cases and 7.6 million cancer deaths occurred in 2008 (2). One quarter of this global burden was located in Europe (3).

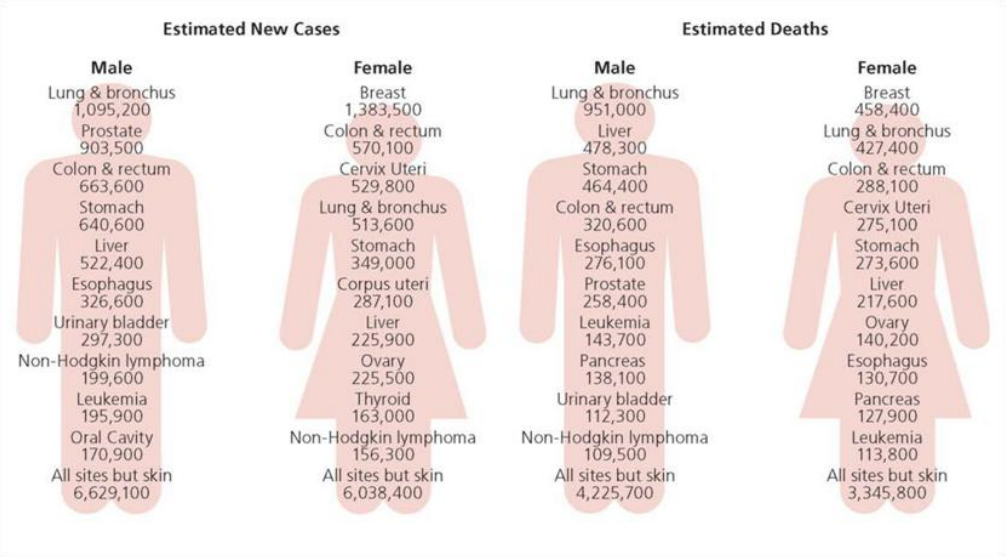


Figure 1.1: Estimated new cancer cases and deaths worldwide for leading cancer sites. GLOBOCAN, 2008. Source: Jemal A. et al. Global cancer statistics. CA Cancer J.Clin. 2011;61:69-90.

By 2030, the world population is expected to have increased to more than 8 billion. During this same year, approximately 26 million new cancer cases will be diagnosed, and 17 million cancer patients will die (4). Given the magnitude of these trends, we need to better characterize the effects of specific risk factors on cancer development, as well as to improve our efforts to control well known modifiable causes of cancer such as cigarette smoking, physical inactivity or poor nutrition (1).

1.1.1.2 Cancer burden in Spain

In Spain, incidence data come from local population-based cancer registries that represent around 30% of the total population (5). Twelve of these registries (Zaragoza, Navarre, Tarragona, Murcia, Granada, Basque Country, Mallorca, Albacete, Asturias, Canary Islands, Cuenca, Girona) contribute to the Cancer Incidence in Five Continents series edited by IARC (6). Based on these series, the GLOBOCAN project estimated

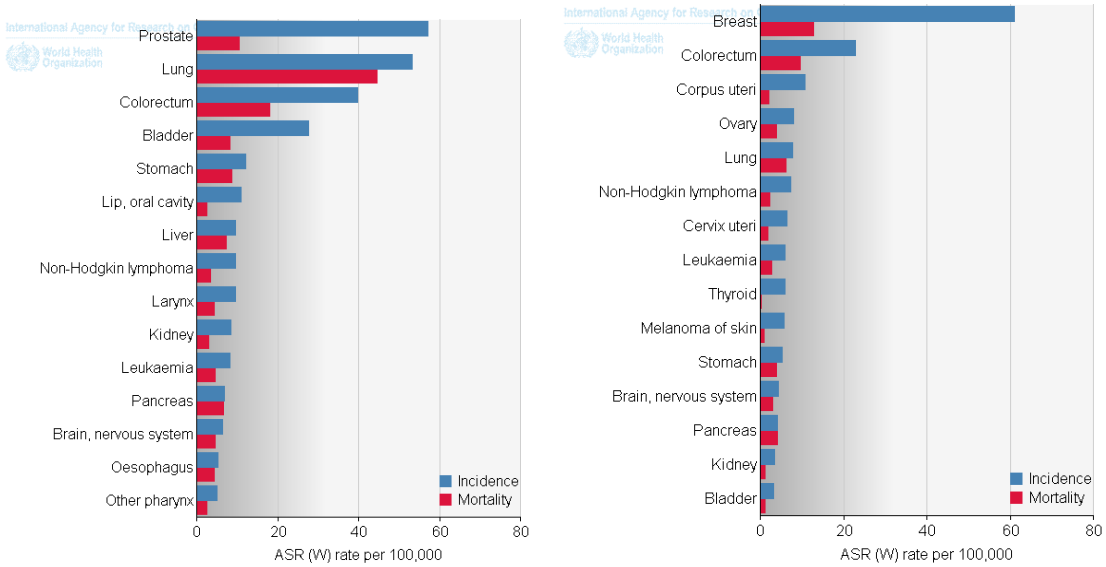


Figure 1.2: Estimated new cancer cases and deaths in Spain for leading cancer sites. Source: Globocan 2008.

that around 196,902 cancers (excluding non-melanoma skin cancer) occurred in this country during 2008. The most frequent locations were prostate, lung and colorectum in men; and breast, colorectum and corpus uteri in women. During this same year, lung and colorectal tumors contributed to 40% of all cancer deaths in men, while those of the breast and lung caused most cancer deaths in women.

1.1.1.3 Cancer burden in the US general population and in American Indian communities

In the US, the American Cancer Society, the Centers for Disease Control and Prevention and the North American Association of Central Cancer Registries provide annual estimations on cancer occurrence and trends. According to these estimations, approximately 1.6 million new cancer cases and 600,000 cancer deaths occurred in 2012.

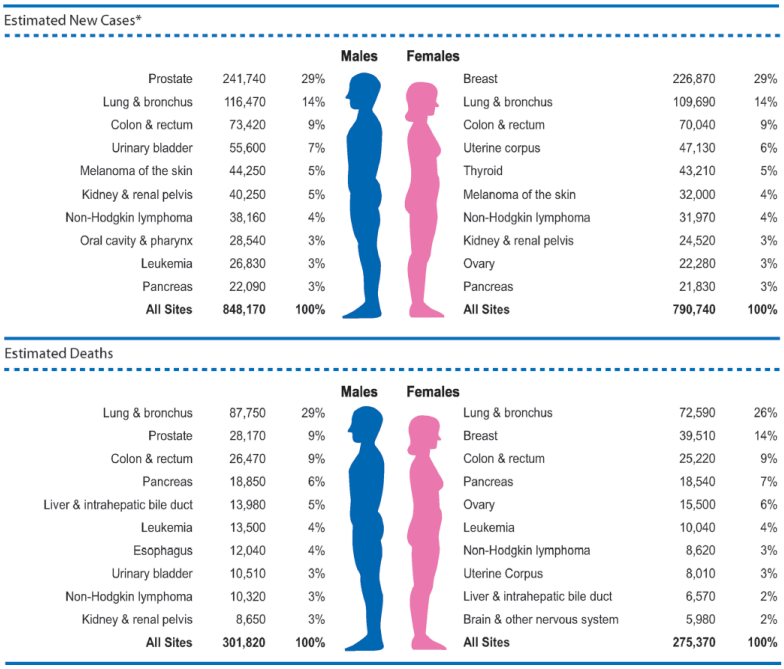


Figure 1.3: Ten leading cancer types for the estimated new cancer cases and deaths by sex, United States, 2012. Source: Siegel R. et al. Cancer statistics, 2012. CA Cancer J.Clin. 2012;1:10-29.

The three most commonly diagnosed types of cancer in men were those of the prostate, lung/bronchus, and large intestine; while in women breast, lung/bronchus and colorectal cancers were the most frequent. Four sites accounted for more than 25% of all cancer deaths: lung/bronchus, prostate and colorectum in men, and lung/bronchus, breast and colorectum in women.

Wide regional variation is characteristic of American Indian cancer surveillance and it is difficult to provide a comprehensive picture of the global burden of cancer in their communities. Studying these populations is difficult because of racial misclassification in medical charts and important disparities associated with lack of health care coverage and

low socioeconomic status. According to the last estimates from the Office of Minority Health (US Department of Health and Human Services), in 2009 the incidence of cancer was 0.8 times lower in American Indians than in Non-Hispanic Whites (7). However, certain types of cancers such as those of the liver, stomach or colorectum, were twice as frequent in American Indians than in the American general population (8). Complex factors are probably contributing to racial disparities, including a range variation in the prevalence of behavioral risk factors for cancer such as tobacco smoke, physical inactivity, obesity or excessive alcohol consumption; access to high-quality screening or treatment. Despite this unequal burden, few epidemiologic studies have focused in this racial group, and the specific cancer risk factors in these populations are unknown (8).

1.1.2 Environmental factors in cancer development

Genes are absolutely not our fate

-Craig Venter-
(1946 -)

Cancer is a chronic disease caused by environmental factors and their interaction with genes. It has been estimated that around 90-95% of cancer cases are directly attributable to environmental and lifestyle risk factors, while only 5-10% have an hereditary origin (9). However, despite remarkable advances in cancer research during the last century, very little is known about the exact environmental risk factors affecting many tumors (10).

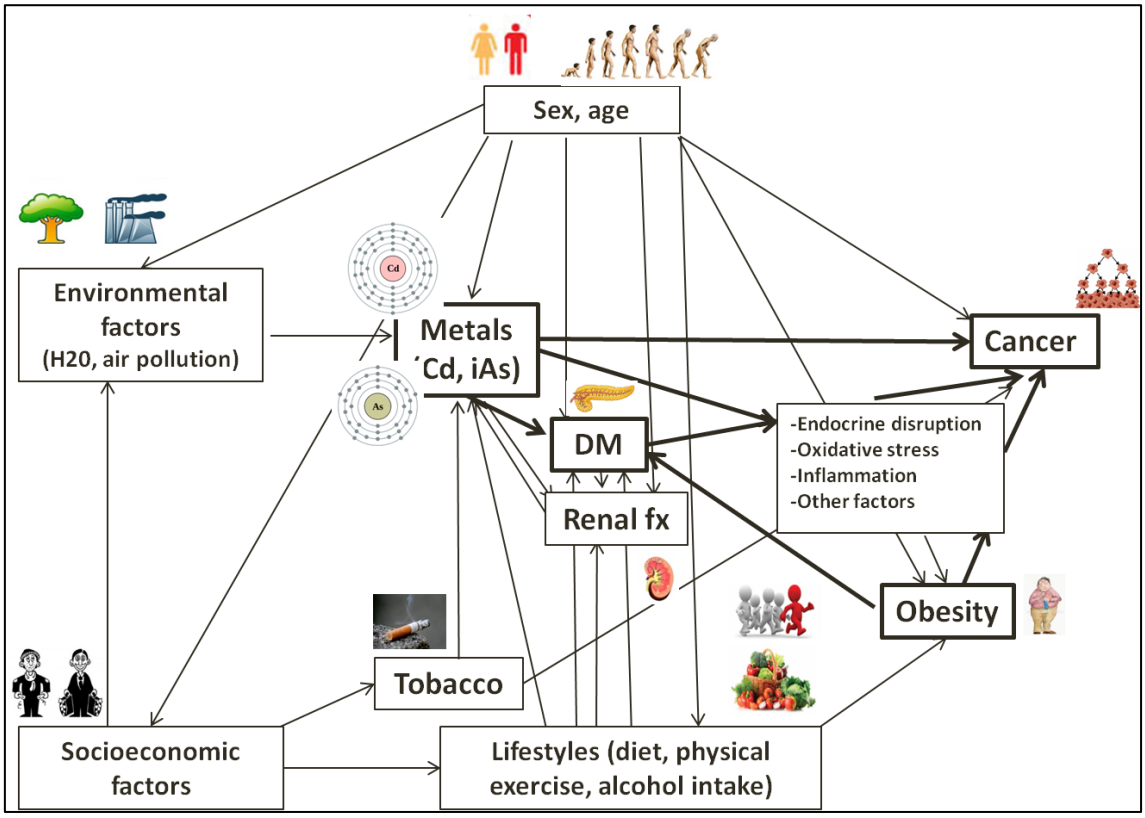


Figure 1.4 Conceptual framework showing the complex relationship between the different studied environmental and lifestyle factors and cancer risk.

The importance of the environment in cancer development was first revealed by migration studies showing that cancer rates in migrants from low cancer incidence countries soon converged to that of the new country (11;12). Later, studies on monozygotic and dizygotic twins have confirmed that inherited genetic factors make a small

contribution to the risk of most cancers types and have shown that even when strong heritable factors exist, the environment acts as the overwhelming contributor to cancer development (13).

Exposure to environmental hazards increases the risk of cancer because they are able to interfere with biological processes. As examples, some agents can alter DNA through genetic or epigenetic changes, leading to activation of oncogenes or inactivation of tumor suppressor genes. Progressive accumulation of mutations in these genes will promote transformation of cells to a malignant state. Other environmental contaminants can damage the immune system breaking the equilibrium that allows the internal environment to control survival and proliferation of mutated cells. Finally, certain agents may also affect the synthesis and function of internal hormones, which maintain the normal function of numerous biologic processes.

1.2 Metals and cancer

1.2.1 Arsenic

Arsenic is a naturally occurring element widely distributed in the earth´s crust. It is present in two main forms: inorganic (arsenite and arsenate) and organic (arsenobetaine, arsenosugars…) compounds. The sources of exposure, biotransformation in the human body and toxicity of inorganic and organic arsenic differ substantially.

1.2.1.1 Exposure

Main sources of inorganic arsenic exposure in the general population include drinking water and certain foods (e.g. rice, grains or juices) (14;15). In seafood, arsenic is mainly found in its less toxic organic form arsenobetaine (16), although some seafood species are also rich in arsenosugars and these can be metabolized to several inorganic arsenic species, mainly DMA.

Inorganic arsenic is released to water from natural erosion of rocks or from agricultural and industrial processes. Additionally, certain anthropogenic sources (i.e. nonferrous metal mining, pesticide application or coal combustion) can also contaminate water with arsenic. The current safety standard for arsenic in drinking water established by the World Health Organization, the US Environmental Protection Agency (USEPA) and the European Union is 10 µg/L (17;18), but there are concerns that this limit may be too high to protect human populations from excess cancer risk (19).

1.2.1.2 Specific sources of arsenic in American Indian communities

In the US, inorganic arsenic exposure through drinking water disproportionately affects certain rural communities from the Western states, including Native Americans (20). Participants in the Strong Heart Study relied either on small public water systems or on private wells. In Arizona and North and South Dakota, arsenic concentrations in public drinking water systems for the studied communities at the time of the study ranged from <10 to 61 µg/L in Arizona and from <10 µg/L up to 21 µg/L in North and South Dakota. Levels in private wells are not known but given arsenic concentrations in groundwater in

these regions (20), it is likely that levels exceeded 10 and even 50 µg/L. As estimated for other populations with arsenic levels in drinking water <10 µg/L, we expect that diet is the main source of arsenic exposure in Oklahoma (21).

1.2.1.3 Biotransformation

Both organic and inorganic arsenic are well absorbed in the gastrointestinal tract, and then widely distributed in the body. There are very few data on the availability of arsenic after inhalation or dermal exposure, although it is estimated that around 75-85% is absorbed after inhalation and <1% after dermal exposure (22). Once absorbed into the body, organic arsenic is not metabolized but is rapidly excreted in the urine (23). Controversially, inorganic arsenic is methylated into monomethylarsenate (MMA) and dimethylarsenate (DMA) which are excreted in urine together with organic arsenic. This methylation is considered to be critical in arsenic toxicity. The average distribution of arsenic metabolites in urine is approximately of around 10-30% for inorganic arsenic (arsenate and arsenite), 10-20% for MMA and 60-80% for DMA (24). Although the excretion pattern for each individual is fairly constant over time, inter-individual differences in the methylation process may influence the proportion of inorganic and organic concentrations in urine. Smoking and drinking alcohol seem to reduce the efficiency of the second methylation step resulting in a higher MA/DMA ratio in urine. Men also tend to have higher MA/DMA than women. Finally, there is some suggestion that nutritional deficiencies, such as folate deficency, can modify arsenic methylation capacity (25).

1.2.1.4 Urine arsenic as a biomarker of exposure

Urinary arsenic is the biomarker of choice for epidemiological studies (23). This is because compared to other biomarkers such as hair, toenail or blood, urine arsenic gives the possibility to assess arsenic biotransformation, as reflected by arsenic species in urine samples. Additionally, arsenic in urine shows a good correlation with levels in drinking water (26). Generally levels of overall arsenic in the urine of non-exposed populations are <50 µg/L (23), although these can vary related to arsenic levels in drinking water and to seafood intake. Increased arsenic clearance is seen in males (27;28), smokers (27) and

people having lower BMI (29), while decreased (30) or similar levels (27) have been found with increasing age.

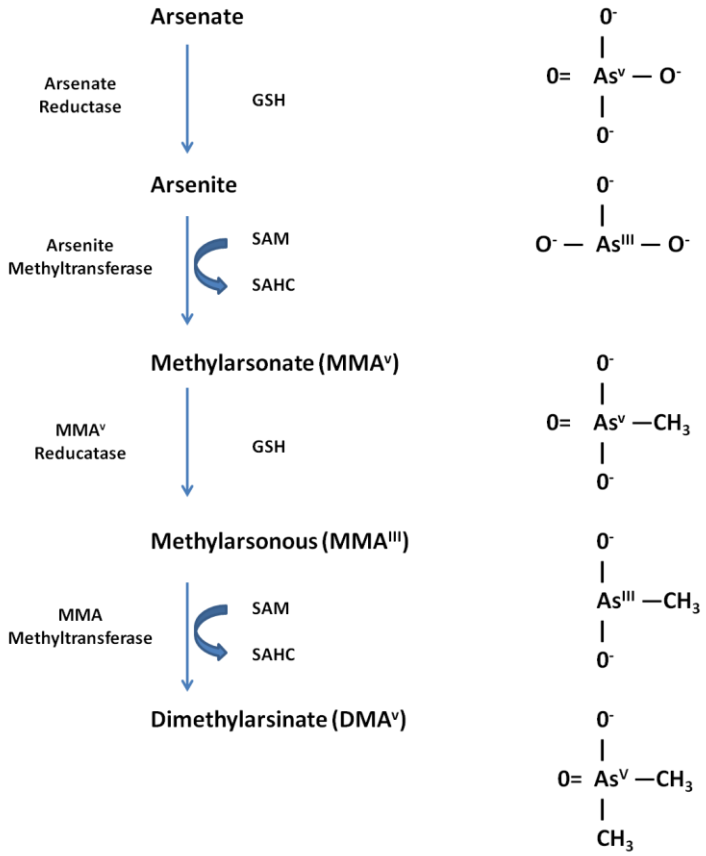


Figure 1.5: Main steps on arsenic metabolism.

1.2.1.5 Health effects

Inorganic arsenic is generally more harmful for human health than its organic forms. Long-term exposure to inorganic arsenic has been associated with skin lesions (31), cardiovascular disease (32), diabetes (33;34), developmental (35) and reproductive problems (36). Additionally, arsenic exposure can lead to cancer development.

Based on human epidemiological data, the International Agency for Research on cancer (IARC) established a causal role for arsenic on skin, lung and bladder cancers, and showed suggestive although limited evidence for liver, kidney and prostate cancers (37). Key epidemiological evidence came from populations chronically exposed to high arsenic

levels in drinking water (>150 µg/L) in southwestern Taiwan (38), Bangladesh (39), northern Chile (40), and Argentina (41).

The evidence at low-moderate arsenic levels remains limited for most cancers, and the few epidemiological studies that have addressed this issue are mostly ecological or retrospective (42).

1.2.1.6 Mechanistic evidence for arsenic carcinogenesis

Evidence for carcinogenicity exists for several arsenic species including inorganic arsenic (arsenite and arsenate) and methylated species (trivalent and pentavalent MMA and DMA), with trivalent arsenic species generally believed to be more toxic. However, the exact mechanisms for arsenic carcinogenicity remain unclear. A major challenge has been to develop animal models for arsenic carcinogenesis, with transplacental models being essential to advance our understanding (43). Some of the accepted mechanisms involve genetic and epigenetic changes, oxidative stress, enhanced cell proliferation, mitochondrial damage and modulation of gene expression (44-47).

1.2.2 Cadmium

1.2.2.1 Exposure

Cadmium pollution in soil, air and water is ubiquitous due to its use in industrial products (batteries, coatings and plastic stabilizers), contamination of phosphate fertilizers, and release from motor vehicle fuel combustion and tire way (48). Soil contamination is a major health problem because leafy/root vegetables and grains bio-concentrate cadmium (49), resulting in major sources of this metal exposure through diet and smoking (50). For this reason, in smokers, tobacco is the main source of cadmium exposure, while in the non-smoking general population living in non-polluted settings, diet is the main source of exposure (51).

1.1.1.1.1 Specific sources of cadmium in American Indian communities

In Native American populations from North America, relevant sources of cadmium exposure include living in the vicinity of contaminant plants and mining areas (52;53) and surface-dust in jewelry-making homes (54). Small scale motor vehicle repair is another activity that could be relevant for cadmium exposure in these communities (55).

1.2.2.2 Biotransformation

Around 25-50% of inhaled cadmium is absorbed (56). Estimated rates of absorption after ingestion are 5% for men and 10% for women (56). This variability among sexes is attributed to the lower iron stores in women, as cadmium and iron are both absorbed in the intestine by the divalent metal transporter 1 (57). In the human body, cadmium binds to metallothionein, believed to play an important role in preventing cadmium toxicity, but also responsible for long-term bioaccumulation of this metal in different tissues, particularly in kidney and liver (58). Only a small fraction of cadmium (~5%) is excreted, while the rest accumulates in the body (56).

1.2.2.3 Urine cadmium as a biomarker of exposure

Urine cadmium is often used as a measure of long-term exposure, as it is proportional to the body burden of this metal. In this sense, age has always been considered one of the most important determinants of urine cadmium concentrations (56). However, new studies suggest that at low levels of exposure, cadmium may be highly influenced by recent intake (59). Generally, levels of cadmium in the urine of non-exposed populations are < 1µg/g creatinine, although these can be much higher in smokers (56). Variations in diuresis or glomerular filtration may affect urine cadmium (59), particularly in individuals with kidney damage (56).

1.2.2.4 Health effects

Kidneys and bones are major targets for cadmium toxicity. Dose-response assessment using early markers of kidney damage, identify urinary cadmium limits of departure for kidney effects between 1.5 and 3.2 µg/g creatinine (60), similarly to those described for bone injury (61). Long-term cadmium exposure has also been associated with increased risk of reduced bone mineral density and bone fractures (62), renal damage (63), hypertension (64;65), diabetes (66), peripheral artery disease (67;68) and coronary heart disease (69).

Cadmium was classified as a human carcinogen by IARC in 1993 (70). Since then, results from epidemiological studies in cadmium polluted areas and occupationally exposed populations have strengthen the evidence that cadmium exposure increases the risk of lung cancer (71-73). In occupationally exposed women, some (74) but not all (71) studies have found an increased risk of breast cancer. For prostate cancer, although a number of early epidemiological studies reported an increased risk among cadmium workers (75;76), the evidence is still inconsistent. Some studies also support occupational cadmium exposure to be a risk factor for kidney (77) and pancreatic cancer (78).

Less is known about the carcinogenic effects of cadmium at low-moderate levels of exposure. In the Third National Health and Nutrition Examination Survey (1988-1994), urine cadmium was associated with overall cancer mortality over 13.5 years of follow-up (79). In men, cadmium was associated with cancers of the lung, pancreas and non-Hodking lymphoma but not with prostate cancer; while in women it was associated with cancers of the lung, ovaries, uterus and leukemia, but not with breast cancer. Cadmium exposure,

however, has been associated with breast cancer in women from general populations in Sweden (80) and the US (81;82).

1.2.2.5 Mechanistic evidence for cadmium carcinogenesis

Proposed mechanisms for cadmium carcinogenicity include oxidative stress (83-86), inhibition of DNA repair systems (87-89), apoptosis (90), epigenetic modifications affecting gene transcription (83;91) or endocrine disruption (92). It has been suggested that cadmium may act differently in different tissues (93), through differential induction of metallothionein expression (94). In human airway epithelial cells, cadmium can promote inflammation through cytokines (95) and increased reactive oxygen species formation (96). In vitro, chronic exposure of human pancreatic duct epithelial cells to cadmium results in malignant cell transformation with increased secretion of metalloproteinases, increased invasiveness and colony formation (97).

1.3 Obesity

Except for smoking, obesity is now the number one preventable cause of death

- Dr. C. Everett Koop-
(1916-2013)

1.3.1 Global burden of obesity

The prevalence of overweight and obesity is increasing worldwide at an alarming rate, as a result of changes in dietary patterns and reductions in physical activity. Over the past three decades, the prevalence of obesity has doubled from 6.4% to 12%, and the prevalence of overweight has changed from 24.6 to 34.4%. If recent trends continue, it is estimated that by 2030, 38% of the overall population will be overweight and 20% obese (98).

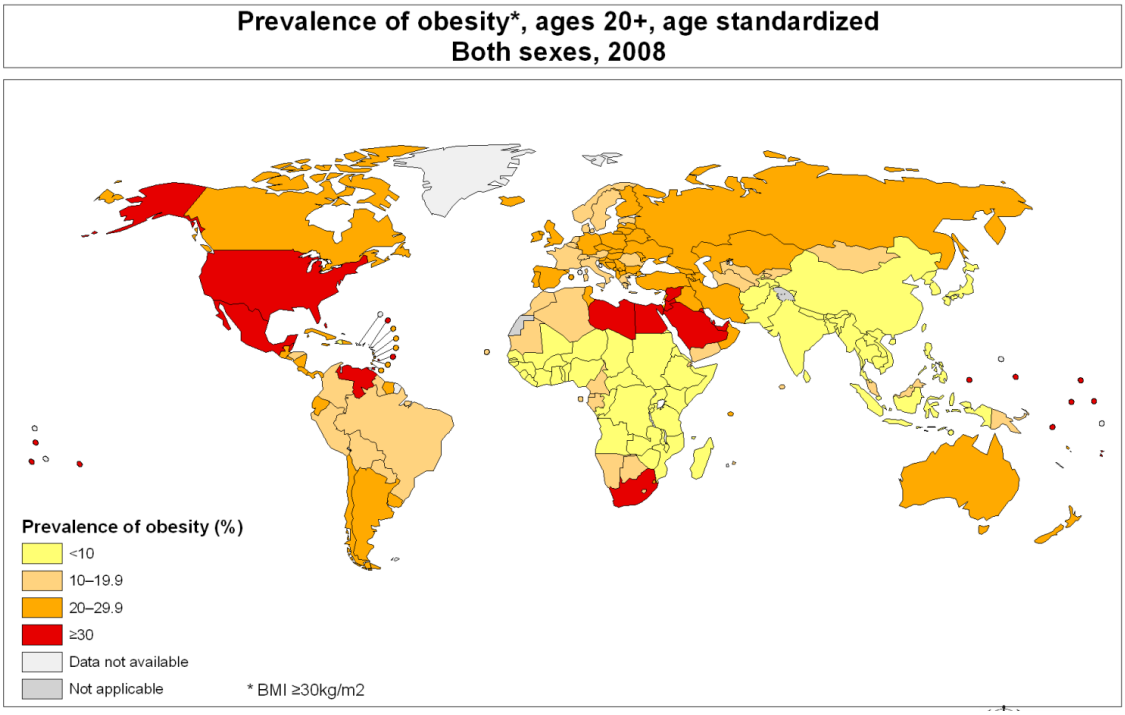


Figure 1.6 : Prevalence of obesity (BMI≥ 30 kg/m^2) in adults in 2008. Source: World Health Organization, 2011.

The prevalence of obesity differs remarkably across countries and it has reached epidemic magnitudes in some regions of the world. Very high prevalence of adult obesity can be observed in North America, Mexico or some countries of the Arab Gulf. In Europe, the highest prevalence is observed in eastern states and in some specific western countries such as the UK or Spain.

1.3.2 Measures of obesity in epidemiological studies

BMI has traditionally been the most commonly used marker of obesity in epidemiological studies, even if it has a poor sensitivity in finding excessive adiposity as it does not account for differences in body fat and muscle mass (99;100). Consequently, people with too much adiposity can be misclassified as non-obese based on their BMI (99). Waist circumference (WC) and waist-to-hip ratio (WHR) are central adiposity markers able to capture excesses in adipose tissue in subjects with normal BMI, and have been shown to be better risk predictors of obesity-related syndromes and all-cause mortality (100).

Other methods to estimate total body fat mass such as DXA (Duel-energy X-ray Absorptiometry), CT (Computed Tomography) or MRI (Magnetic Resonance Imagining) are generally too costly and complex to be used in large epidemiological studies (101).

1.3.3 Health effects

According to the World Health Organization, overweight and obesity account for more than 2.8 million deaths each year and 2.3% of global DALYs. Overweight and obesity have been associated with the risk of diabetes (102), coronary heart disease (103), high blood pressure (104), high cholesterol (104), stroke (103) and obesity-hypoventilation syndrome (105).

In 2002, IARC concluded that people who are overweight or obese are at increased risk of certain tumors including those of the colorectum, breast (in postmenopausal women), oesophagus, endometrium and kidney. Since then, many epidemiological studies have been published showing a link between obesity and the risk of liver (106), gallbladder (107), bladder (108), ovarian (109), thyroid (110) or aggressive prostate tumors (111). Overall, it has been estimated that overweight and obesity could account for up to 15-20% of all cancer cases (112).

1.3.4 Mechanistic evidence for the link between excessive body fatness and cancer risk

Obesity is associated with hyperglycemia and insulin resistance (113). Both factors promote the synthesis and biological activity of IGF-1, which in turn can stimulate cell growth proliferation and survival through the PI3K/Akt pathway (113). Additionally, insulin and IGF-1 influence the bioavailability of sex steroids by reducing the hepatic concentrations of sex-hormone binding globulin (SHBG) and this mechanism is thought to play a major role in the genesis of certain hormone-dependent tumors (101). In this sense, reductions in SHBG lead to increased levels of both estradiol and testosterone in women. In men, only estradiol levels are increased while SHBG reductions inhibit testosterone synthesis. Additionally, in severely obese men, the increased levels of estradiol activate the hypothalamic estrogen receptors further inhibiting the gonadotropic stimulation of testicular testosterone production (114).

Emerging evidence points to an important role of local inflammation on cancer development. In obesity, adipocyte hypertrophy is associated with increased levels of free fatty acids that stimulate the NF-kB pathway in macrophages. Through this mechanism, the activated macrophages release tumor necrosis factor which facilitates the release of more fatty acids creating a paracrine loop that contributes to maintaining the proinflammatory state necessary for tumor carcinogenesis (113). Increased levels of leptin, the main pro-inflammatory adipokine secreted by the adipose tissue, and decreased levels of adiponectin, a hormone with important antitumoral effects, have also been proposed as important factors in the development of many cancers, including those of the breast and prostate (115;116). Finally, increased levels of VEGF and other proangiogenic factors (i.e. PAI-1) have shown to promote tumor growth and facilitate the metastatic spread of cancer cells (113).

1.4 Diabetes and cancer

Three horses draw the diabetic chariot and their names are diet, exercise and insulin.

-Elliott Proctor Joslin-
(1869-1962)

1.4.1 Global burden of disease

Diabetes mellitus is a leading causes of death and disability worldwide (117). In 2011, an estimated 366 million people had diabetes, and according to current projections from the International Diabetes Foundation, by 2030 this number will have risen to 552 (118). Reasons for this dramatic increase are similar to those described for obesity and include aging, urbanization, sedentary lifestyles and poor nutrition.

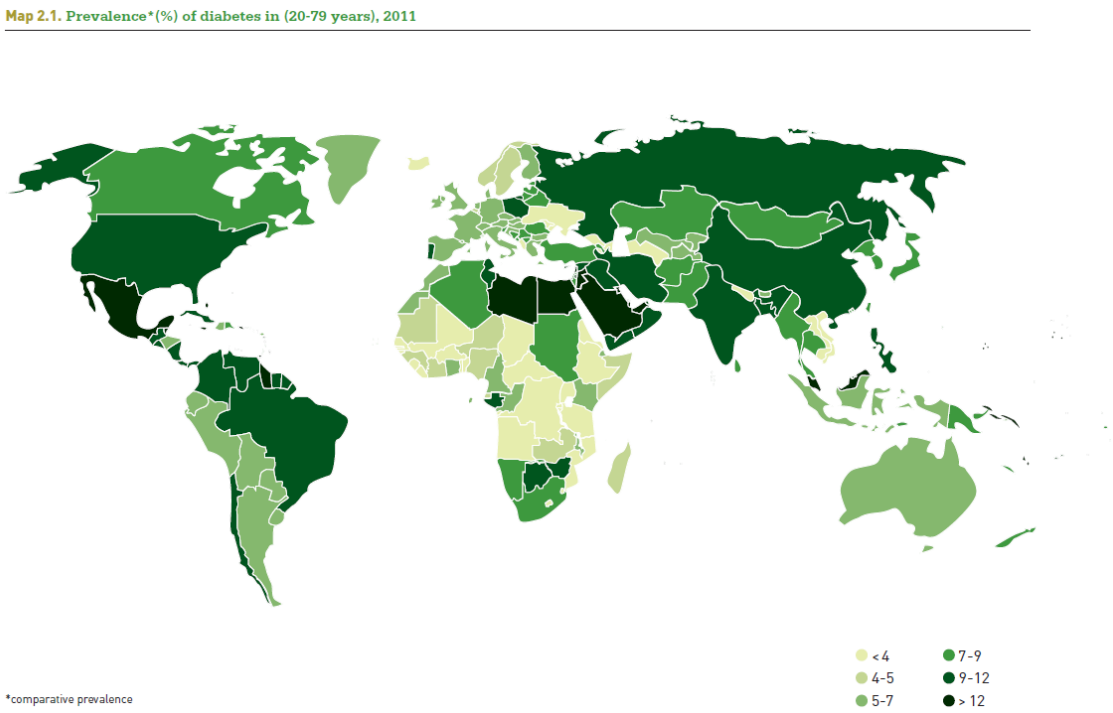


Figure 1.7 : Prevalence of diabetes in adults in 2011. Source: Diabetes Atlas, 2012.

Around 80% of diabetic individuals live in low- and middle- income countries, where diabetes is already an epidemic (118). Countries like China, India, US, Russia, Brazil, Bangladesh or Mexico face the greatest burden of diabetes. In Europe, with an estimated 52.6 million cases of diabetes in 2011, the countries with the highest prevalence are located in the eastern regions, including Poland, Belarus or Latvia. Conversely, the

highest number of people with diabetes are observed in the western countries like Germany, Italy or Spain (119).

1.4.2 Health effects

According to the International Diabetes Foundation, in 2011 around 4.6 million adults died from diabetes or its complications, and around 50% of these deaths occurred in individuals under the age of 60 (118). Diabetes has been associated with an increased risk of microvascular complications (120), heart failure (121), stroke (121), peripheral artery disease (121), chronic kidney disease (122) and obstructive sleep apnea (123).

Substantial evidence increasingly supports that type-2 diabetes is a risk factor for the development of numerous types of cancers, including those of the pancreas, liver, stomach, colorectum, kidney, bladder, postmenopausal breast and endometrium (124;125). In contrast, a reduced incidence of prostate cancer has been observed in diabetic individuals (126). Still, a number of important questions remain unanswered. First, it is unclear whether the association between diabetes and cancer can be attributed to the existence of common risk factors for both diseases (i.e.: age, obesity, lack of physical activity), or to the direct effect of insulin resistance and its compensatory hyperinsulinemia and hyperglycaemia. Second, the latency period from diabetes exposure to cancer risk is unknown. Because changes in glucose concentrations, insulin sensitivity or insulin secretion can precede diagnosis of diabetes to up to 6 years (127), the increased risk of cancer could also predate clinical diagnosis of diabetes (128). Finally, anti-diabetes medication may also modulate the risk of cancer, and more research is needed to disentangle the effects of diabetes from those derived from its treatment (125). This is even more difficult if we take into account that most diabetic patients are treated with more than one glucose-lowering drug at the same time (129), and that treatment schemes change over the course of the disease according to its severity (130).

1.4.3 Mechanistic evidence for the link between diabetes and cancer risk

Several mechanisms linking diabetes and cancer risk have been described including hyperinsulinemia, hyperglycemia and chronic inflammation (125). Hyperglycemia induces oxidative stress and activation of the renin-angiotensin system, and both mechanisms can produce dysregulation of cholesterol metabolism and endothelial dysfunction (131). Insulin can promote cancer through its direct effect on cell proliferation and survival, or indirectly by reducing the hepatic production of IGF binding protein with resultant increased circulating levels of IGF-1. In malignant cells, the A isoform of the insulin receptor is expressed predominantly and its activation can stimulate insulin-mediated mitogenesis (132). Additionally, many cancers express the IGF-1 receptor, which has shown to have a more mitogenic and transforming activity than the insulin receptor (125) . Finally, low-grade chronic inflammation, a common feature in subjects with type 2 diabetes, can also induce cancer development through similar mechanisms as those explained in section 1.3.4.

2 HYPOTHESIS AND OBJECTIVES

HYPOTHESIS 1:

1.1 Low to moderate exposure to inorganic arsenic is associated with overall cancer mortality and with mortality from cancers of the lung, liver, prostate and kidney.

HYPOTHESIS 2:

2.1 Low to moderate exposure to cadmium is associated with overall cancer mortality, with mortality from tumors associated with tobacco smoke (including lung) and with mortality from cancers of the kidney and prostate.

HYPOTHESIS 3:

3.1 Measures of central adiposity (waist circumference and waist-to-hip-ratio), but not BMI are associated with an increased risk of breast cancer in premenopausal women.

3.2 Measures of central (waist circumference and waist-to-hip-ratio) and general (BMI) adiposity are associated with an increased risk of breast cancer in postmenopausal women.

3.3 Changes in weight since age 20 are associated with an increased risk of breast cancer both in pre and postmenopausal women.

3.4 The effects of the studied anthropometric factors on cancer risk may differ by intrinsic subtypes.

3.5 Measures of central (waist circumference and waist-to-hip-ratio) and general (BMI) adiposity are associated with an increased risk for advanced prostate cancers and a decreased risk for non-aggressive prostate tumors.

HYPOTHESIS 4:

4.1 Type-2 DM is associated with an increased risk of breast cancer in postmenopausal women.

4.2 Type-2 DM is associated with a decreased risk of prostate cancer, and this decreased risk becomes stronger with longer diabetes duration.

4.3 Metformin may reduce the risk of breast and prostate cancer, while insulin and insulin secretagogues may increase this risk.

OBJECTIVE 1:

General objective: To evaluate the prospective association between exposure to heavy metals and cancer mortality in adults 45-75 years of age from 3 American Indian communities who participated in the Strong Heart Study from 1989-1991 through 2008.

Specific objectives

Objective 1.1: To evaluate the association of urinary inorganic arsenic with all cause and site-specific cancer mortality in Native Americans who participated in the Strong Heart Study.

Objective 1.2: To evaluate the association of urinary cadmium with all-cause and site-specific cancer mortality in Native Americans who participated in the Strong Heart Study.

Objective 1.2.1: To assess the role of cadmium as a possible mediator in the association between tobacco smoke and cancer mortality.

OBJECTIVE 2:

General objective: To estimate the effect of obesity, fat distribution and weight changes in adulthood over the risk of hormone-dependent tumors in adults 20-85 years of age who participated from 2008 to 2013 in a Spanish multicenter population-based case-control study (MCC-Spain).

Specific objectives

Objective 2.1: To study the association between several self-reported and measured anthropometric variables (age at maximum height, age at maximum weight, weight at ages 20 and 45, changes in weight since age 20, body-mass-index 1, waist circumference and waist-to-hip ratio) and the risk of breast cancer, overall and by intrinsic subtypes, in MCC-Spain.

Objective 2.2: To study the association between several self-reported and measured anthropometric variables (age at maximum height, age at maximum weight, weight at ages 20 and 45, changes in weight since age 20, body-mass-index 1, waist circumference and waist-to-hip ratio) and the risk of prostate cancer, overall and Gleason score at diagnosis, in MCC-Spain.

OBJECTIVE 3:

General objective: To evaluate the association between diabetes, diabetes treatment and diabetes duration with the risk of hormone-dependent tumors in adults 20-85 years of age who participated in a Spanish multicenter population-based case-control study (MCC-Spain) from 2008 to 2013.

Specific objectives

Objective 3.1: To study the association between self-reported diabetes, diabetes duration and the risk of postmenopausal breast cancer, overall and by intrinsic subtypes, in MCC-Spain.

Objective 3.2: To study the association between self-reported diabetes, diabetes duration and diabetes treatment and the risk of prostate cancer, overall and by Gleason score at diagnosis, in MCC-Spain.

3 METHODS

3.1 Methods for objective 1

3.1.1 Study population

The Strong Heart Study (SHS) is the largest epidemiologic study of cardiovascular disease in American Indian populations ever undertaken. During 1989-1991, 4,549 men and women 45-75 years of age were recruited from 13 tribes and communities in three geographic regions: an area near Phoenix (Arizona), the southwestern area of Oklahoma, and western and central North and South Dakota.

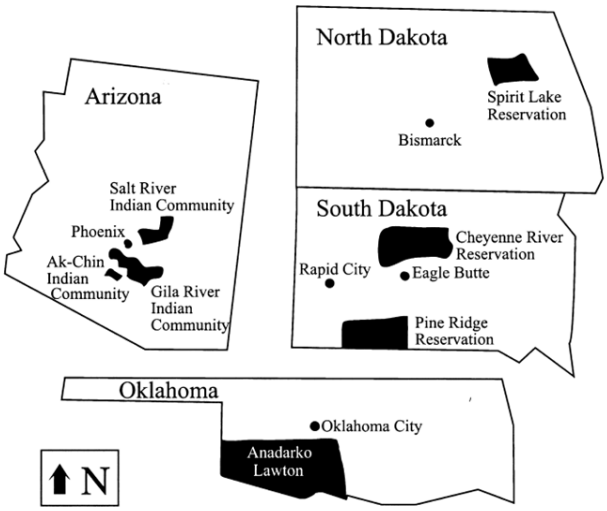


Figure 3.1: Map of the Strong Heart Study Communities.

The aim was to recruit approximately 1,500 participants per region. In Arizona and Oklahoma every eligible person was invited; in North/South Dakota a cluster sampling technique was used (133). Overall, the baseline participation rate was 62%. The Strong Heart Study protocol and consent form were approved by the Institutional Indian Health Service Review Boards and by the participating Indian communities. Informed consent was obtained from all participants.

The Strong Heart Study was initially designed to study the prevalence of known or suspected cardiovascular disease risk factors in American Indians, and to assess their influence on cardiovascular morbidity and mortality (http://strongheart.ouhsc.edu/). However, the long follow-up of Strong Heart Study participants has allowed us to secondary evaluate the influence of some of this risk factors on cancer mortality.

3.1.1.1 Study sample

From the initial population, we excluded participants with insufficient urine for metal analyses and participants with missing values in potential important confounders, leaving 3,935 participants for arsenic and 3,792 for cadmium analyses. The smaller sample size in the case of cadmium is explained by the exclusion of participants with no information on cigarette pack-years, as tobacco smoking is one of the most relevant potential confounders in the association between cadmium and cancer risk. In the case of arsenic, adjustment for cigarette pack-years was only performed as a sensitivity analyses, and so individuals with no information on this variable were included in the main results.

3.1.2 Exposure assessment

Spot urine samples were collected in polypropylene tubes, frozen within 1 to 2 hours of collection, shipped buried in dry ice and stored at -70°C in the Penn Medical Laboratory, MedStar Research Institute, Washington, DC for up to 18 years. The freezers have been operating under a strict quality control system to guarantee secure sample storage. For analysis, urine samples were thawed. From each urine sample, up to 1.5 mL for arsenic and 1.0 mL for cadmium determinations were transferred to a small vial, transported on dry ice to the Trace Element Laboratory at Graz University, Austria and stored at -80°C.

Inorganic arsenic (arsenite, arsenate), monomethylarsonate (MMA), dimethylarsinate (DMA), and arsenobetaine plus other arsenic cations were measured using anion-exchange high-performance liquid chromatography (Agilent 1100 HPLC, Agilent Technologies, Waldbronn, Germany) coupled with inductively coupled plasma mass spectrometry (Agilent 7700x ICPMS). The limits of detection were 0.1 µg/L for total arsine and 0.5 µg/L for arsenite, arsenate, methylarsonate (MMA) and dimethylarsinate (DMA). The percentages of participants with concentrations below the limit of detection were 5.3% for inorganic arsenic, 0.7 % for MMA, 0.03 % for DMA and 2.1% for arsenobetaine plus other cations. For participants with concentrations below the limit of detection we divided the corresponding limit of detection by the square root of two. The inter-assay coefficients of variation were 6.0% for inorganic arsenic, 6.5% for MMA, 5.9% for DMA and 6.5% for arsenobetaine plus other cations. Arsenobetaine concentrations

were very low (median 0.76 µg/L, interquartile range 0.47-1.70 µg/L), confirming that seafood intake is rare in the Strong Heart Study population.

Urine cadmium concentrations were measured using inductively coupled plasma-mass spectrometry (ICPMS). The limit of detection for urine cadmium was 0.015µg/L with only one participant having values below this limit. The inter-assay coefficient of variation was 8.7%. Urine cadmium concentrations were corrected for molybdenum oxide interference using the formula [Cd]corr = [Cd]-0.0016*[Mo] (134).

3.1.3 Data collection and variables definition

Study visits were performed by trained and certified examiners following a standard protocol (133), and included a questionnaire, a physical examination and biospecimen collection (blood, urine). Information on sociodemographic factors (age, sex, study region, education), smoking status, alcohol use, reproductive factors in women (menopausal status, parity) and medical history was obtained from the baseline SHS questionnaire (133). Height and weight were measured and body mass index was calculated as weight in kg divided by height in meters squared. Hypertension was defined as mean systolic blood pressure ≥140 mm Hg, mean diastolic blood pressure ≥90 mm Hg, or antihypertensive medication use. Plasma creatinine was measured by an alkaline-picrate rate method. Estimated glomerular filtration rate (eGFR) was calculated from calibrated creatinine, age and sex using the Modification of Diet in Renal Disease Study formula without an ethnicity factor. Diabetes, a major public health problem in the study communities, was defined as fasting plasma glucose ≥126 mg/dL, 2-h post-load plasma glucose ≥200 mg/dL, hemoglobin A1c ≥6.5%, or use of insulin or oral hypoglycemic agents. Methods to measure fasting glucose, 75-g oral glucose tolerance test, and hemoglobin A1c have been described elsewhere (133).

Urine creatinine was measured at the laboratory of the National Institute of Diabetes and Digestive and Kidney Diseases Epidemiology and Clinical Research Branch (Phoenix, AZ, USA) by an automated alkaline picrate methodology (133). To account for urine dilution in spot urine samples, we divided urine arsenic by urine creatinine and expressed the concentrations of total urine arsenic and its species as micrograms per gram creatinine.

3.1.4 Cancer mortality follow-up

Follow-up for mortality was complete for 99.8% of the study population (135). Death certificates were obtained from the State Departments of Health. If the death certificate indicated that an autopsy had been performed, the medical examiner´s report was obtained (133). Death certificate codes were recorded by a single nosologist according to the International Classification of Diseases, 9th Revision (ICD-9) (136). Malignant neoplasm include ICD-9 codes 140 to 208. In addition to total cancer, we evaluated the following specific cancers: esophagus and stomach (ICD-9 150-151), colon and rectum (ICD-9 153-154), liver and intra-hepatic bile ducts (ICD-9 155) gallbladder and extra-hepatic bile ducts (ICD-9 156), pancreas (ICD-9 157), bronchus and lung (ICD-9 162.2-162.9) (referred from now on as lung cancer), breast (ICD-9 174), prostate (ICD-9 185), kidney (ICD-9 189.0) and lymphatic and hematopoietic tissue (ICD-9 200-208). Skin and bladder cancers were not evaluated as the corresponding number of deaths was very small (2 and 1, respectively).

For cadmium analyses, we grouped cancer with sufficient evidence for tobacco smoking according to the IARC (IARC 2012): Lip, oral cavity and pharynx (ICD-9 140-149), esophagus (150), stomach (151), colon and rectum (ICD-9 153-154), liver (155), pancreas (157), larynx (161), trachea, bronchus and lung (162), cervix (180), bladder (188), kidney (189), myeloid leukemia (205).

Time to event was calculated from the date of baseline examination to the date of death or to December 31, 2008 whichever occurred first. The mean follow-up time among participants who did not have a cancer death was 14.6 for participants with arsenic and 16.2 for participants with cadmium determinations at baseline.

3.1.5 Statistical methods

3.1.5.1 Specific methods for objective 1.1

We used the sum of inorganic (arsenite and arsenate) and methylated (MMA and DMA) arsenic corrected by urine creatinine as the biomarker of exposure to inorganic arsenic. Seafood intake in the SHS population was rare (137), as confirmed by very low concentrations of arsenobetaine (median 0.76 µg/L, interquartile range 0.47 and 1.70 µg/L), thus allowing us to use the sum of inorganic and methylated arsenic species as a

biomarker of inorganic arsenic exposure. Urine arsenic concentrations were markedly right-skewed and were log-transformed for statistical analyses.

The prospective association between the creatinine-corrected sum of inorganic and methylated arsenic and cancer mortality (overall and site-specific) was assessed using Cox-proportional hazards models with age as time scale and individual starting follow-up times (age at baseline examination) treated as staggered entries. This approach effectively adjusts for age. The assumption of hazards proportionality was evaluated based on the smoothed association between age and scaled Schoenfeld residuals (138), with no major departures from proportionality. In order to control for region, the non-parametric underlying baseline hazards were allowed to differ by study region using the *strata* command in Stata. We modeled arsenic in 3 ways: (1) tertiles; (2) 80^{th} vs. 20^{th} percentiles of log-transformed arsenic; and (3) restricted cubic splines with knots at the 10^{th} (3.80 µg/g creatinine), 50^{th} (9.68 µg/g) and 90^{th} (24.0 µg/g) percentiles to evaluate potential non-linear relationships. P-values for linear trend were obtained from Wald tests by introducing log-arsenic concentrations as a continuous variable. Departures from linearity in the restricted cubic spline models were evaluated using the Wald test.

All Cox-proportional hazards models initially accounted for region (stratification factor) and age. In addition, we further adjusted for sex, education (<high school, some high school, ≥high school), smoking status (never, former, current), drinking status (never, former, current) and BMI. For breast cancer, models were further adjusted for menopausal status and parity. For kidney cancer, we further adjusted for hypertension (no, yes) and reduced estimated glomerular filtration rate (<60, ≥60 ml/min/1.72m^2) (139).

To evaluate the consistency of the findings across participant subgroups, exploratory analyses for overall cancer mortality included interaction terms for log-transformed sum of inorganic and methylated arsenic concentrations with indicator variables for subgroups defined by age (<55, 55-64, >64 y), sex (men, women), smoking status (never, former, current), diabetes (yes/no), %DMA (tertiles) and %MMA (tertiles) in separate models. Subgroup analyses were not conducted for specific cancer mortality because the number of deaths was too small.

We performed several sensitivity analyses. First, in participants with cigarette pack-year information (N=3,789), we further adjusted for cigarette pack-years. Second, to account for death by causes other than cancer, we estimated proportional hazard regression

models for competing risks according to the method of Fine and Gray (140) using *stcrreg* command in Stata. Third, to reduce the possibility that prevalent cancers at baseline could affect urine arsenic concentrations we repeated the analyses excluding participants during the first 2 years or the first 5 years of follow-up. Fourth, to evaluate the stability of the effect along time we conducted separate analyses for the first and second decades of follow-up. Fifth, to confirm that the findings were not affected by using age as the time scale, we fitted models using calendar time as the time scale and adjusted by age, and tested if the proportional hazard assumption was fulfilled for arsenic. The results of these sensitivity analyses were similar to those of the main analyses (not shown). Finally, we used the following two alternative strategies to account for urine dilution: (1) adjusting for log-transformed urine creatinine in the regression models instead of dividing urine arsenic by urine creatinine concentrations; (2) adjusting urine arsenic concentrations to the overall mean specific gravity in the study population of 1.019 (141). This last analysis was restricted to participants without albuminuria and diabetes because specific gravity is inadequate to adjust for dilution if albumin or glucose are present in urine (142;143).

3.1.5.2 Specific methods for objective 1.2

Urine cadmium concentrations were also right-skewed and log-transformed for statistical analyses. To account for urine dilution in spot urine samples, we divided cadmium by urine creatinine.

Similarly to the analyses for arsenic, the prospective association between creatinine-corrrected cadmium concentrations and cancer mortality was assessed using Cox-proportional hazards models with age as time scale and individual starting follow-up times treated as staggered entries. Cadmium was also modeled in 3 different ways: (1) tertiles; (2) 80^{th} vs. 20^{th} percentiles of log-transformed cadmium; and (3) restricted cubic splines with knots at the 10^{th} (0.40 µg/g creatinine), 50^{th} (0.93 µg/g creatinine) and 90^{th} (2.15 µg/g creatinine). For pancreas cancer, there was only 1 case in the first cadmium tertile and for this reason we combined the first and second tertiles.

All Cox-proportional hazard models initially accounted for age and region. A second model further adjusted for sex, BMI, smoking status and cigarette pack-years. For kidney cancer mortality we also adjusted for hypertension and reduced estimated glomerular filtration rate, and for breast cancer mortality, for parity. In addition, we

adjusted models for postmenopausal status in women, education and drinking status added one by one, with similar results (data not shown).

To evaluate the consistency of our findings across participants' subgroups, exploratory analyses for overall cancer mortality and for smoking-related cancer mortality included interaction terms for log-transformed cadmium with indicator variables for subgroups defined by age, sex, menopausal status, smoking status, pack-years, BMI and urine arsenic concentrations in separate models. We could not conduct interaction analyses for specific cancers due to the relatively small number of events.

Finally, as in the case of arsenic, sensitivity analyses included accounting for competing risks using Stata *stcrreg* command, excluding participants during the first 2 or 5 years of follow-up, conducting separate analyses for the first and second decades of follow-up and using alternative strategies to account for urine dilution.

3.1.5.3 Specific methods for objective 1.2.1

To assess the role of cadmium as a possible mediator in the association between tobacco smoke and cancer mortality we calculated the proportion of additional cases of lung cancer due to tobacco smoking that could be attributed to cadmium exposure, using the method proposed by Lange et al. (144), with bootstrap confidence intervals estimated as bias-corrected and accelerated percentile intervals. According to this method, we first calculated the direct effect of smoking, as measured by pack-years, on cancer (direct pathway) using the Aalen additive hazard model. Then, we calculated the indirect effect using 2 models: 1) a linear regression with cadmium as the dependent variable and number of pack-years as the independent variable and 2) the Aalen additive hazard model for cadmium adjusted for pack-years. The proportion of lung cancer mortality associated with a 10 pack-year increase that can be attributed to cadmium exposure, as measured in urine, was calculated as the ratio of the indirect to the total effect.

3.2 Methods for objectives 2 and 3

3.2.1 Study population

MCC-Spain is a population-based case-control study focusing in frequent tumors and/or tumors with peculiar epidemiological characteristics in Spain (http://mccspain.org/). Histologically confirmed cases of breast (ICD-10: C50, D05.1, D05.7), prostate (ICD-10: C61, D07.5), colon or rectum (ICD-10: C18, C19, C20, D01.0, D01.1, D01.2), stomach (ICD-10: C16, D00.2), oesophagus (ICD-10: C15.5), or chronic lymphocytic leukaemia and small lymphocytic lymphoma (ICD-10: C91.1) were recruited from 22 Spanish public hospitals between 2008 and 2013. Cases were identified through the medical registries of the participating hospitals. All cases were incident and were excluded if they had a previous diagnosis of cancer in the same location under study. Inclusion criteria required that cases had lived for at least 6 months in the one of the study areas and were between 20-85 years of age.

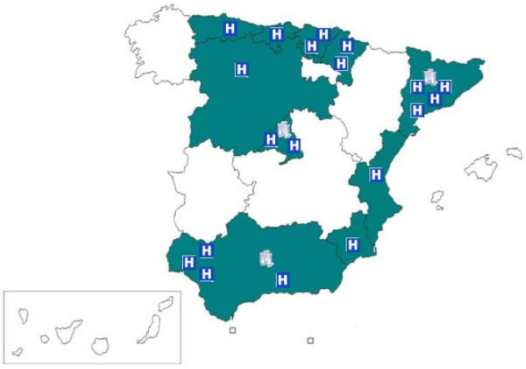


Figure 3.2: Map of the recruiting centers in MCC-Spain.

Population-based controls were randomly selected from lists of primary care health centres within the catchment areas of the hospitals where cases were recruited and contacted by telephone. Controls were frequency-matched to the cases, taking into account age, sex and region. Exclusions were made if patients had any physical or mental disorder that could impede their participation in the study.

Response rates were 53% for healthy controls, 71% for breast cancer cases and 72% for prostate cancer cases. There were no differences in the main socio-demographic variables among those who participated and those who refused to participate. All participants who agreed to participate signed an informed consent and the study was formally approved by the corresponding Ethics Committee of each area. The MCC-Spain

study also followed the declaration of Helsinki and the Spanish Personal Data Protection Act of 1999 (Ley Organica 15/1999 de 13 de Diciembre).

3.2.1.1 Study sample

A total of 1743 breast cancer cases with 1880 matched controls, and 1113 prostate cancer cases with 1460 controls were recruited. The study design intended that all interviewers would collect data from cases and control, but in a small number of participants this was not possible. In addition, not all interviewers registered information for all types of tumors.

For the analyses on anthropometric factors and cancer risk, we excluded those participants whose interviewers had only interviewed cases or controls ($N_{\text{breast cancer cases}}$=104; $N_{\text{breast cancer controls}}$=168; $N_{\text{prostate cancer}}$ =141; $N_{\text{prostate controls}}$ =140). The reason to exclude these participants is that even though a standardized protocol was used to measure the waist and hip circumferences, the measurement process includes an additional source of variability that we wanted to control in the analyses by including the interviewer as a random effect term. Pregnant women (N=15) and participants with missing values in BMI or other important confounders were also excluded from the analyses, leading to a final sample of 1487 breast cancer cases with 1500 controls; and 1018 prostate cancer cases with 1327 controls.

For the analyses on diabetes and cancer risk, we decided not to include premenopausal women because the prevalence of diabetes was very low in this specific subgroup. From the initial 1035 cases of postmenopausal breast cancer with 1252 controls, and 1113 prostate cancer cases with 1460 controls, we excluded those participants with no information on diabetes, diabetes duration or diabetes treatment ($N_{\text{breast cancer cases}}$=20; $N_{\text{breast cancer controls}}$=23; $N_{\text{prostate cancer cases}}$=21; $N_{\text{prostate cancer controls}}$= 51), as well as those with no information in BMI ($N_{\text{breast cancer cases}}$=67; $N_{\text{breast cancer controls}}$=110; $N_{\text{prostate cancer cases}}$=17; $N_{\text{prostate cancer controls}}$= 30) or other important confounders ($N_{\text{breast cancer cases}}$=17; $N_{\text{breast cancer controls}}$=9; $N_{\text{prostate cancer cases}}$=5; $N_{\text{prostate cancer controls}}$=3). However, in a sensitivity analyses, we also included participants with missing values in BMI and imputed their BMI values to check the consistency of the results. In order to reduce the probability of including type-1 diabetic individuals as exposed, we also excluded participants who had been diagnosed of diabetes before the age of 45 ($N_{\text{breast cancer cases}}$=8; $N_{\text{breast cancer controls}}$=11; $N_{\text{prostate cancer cases}}$=10;

$N_{prostate\ cancer\ controls}$=21). Finally, to allow for a minimum latency period and to avoid that the clinical conditions that lead to diabetes and cancer diagnosis could overlap, we also excluded participants diagnosed of diabetes ≤ 1 year before the diagnosis of cancer ($N_{breast\ cancer\ cases}$=7; $N_{breast\ cancer\ controls}$=5; $N_{prostate\ cancer\ cases}$=6; $N_{prostate\ cancer\ controls}$= 14), leading to a final sample of 916 cases and 1094 controls of postmenopausal breast cancer and 1054 cases and 1341 controls of prostate cancer.

3.2.2 Data collection and variables definition

Both cases and controls were interviewed by purposed trained personal, who collected data on socio-demographic factors, health behaviors (such as smoking, dietary patterns, physical activity and screening attendance), gynecologic and obstetric history in female participants, anthropometric factors (age at maximum height; weight at age 20 and age 45; age at maximum weight; weight and height one year prior to the interview), family history of cancer, pre-existing medical conditions (including self-reported diabetes and self-reported age at diagnosis) and treatments received (comprising starting age and current dose). At the end of the questionnaire, interviewers were asked to classify the overall quality of the participant's responses as "fair", "good" or "excellent".

Waist and hip circumferences were measured twice by the interviewer. The waist circumference was measured at the midpoint between the lowest rib and the iliac crest, and the hip circumference around the widest portion of the buttocks. In accordance with standardized protocols, if the first two measures were dissimilar, a third measurement was taken.

Body mass index one year prior to the interview was calculated as the ratio of weight in kilograms to the square of height in meters, and the waist-to-hip ratio computed as the ratio of the circumference of the waist to that of the hips in centimeters. Adult weight gain was defined as the number of kg of difference between self-reported weight at age 20 and self-reported weight one year before the study interview.

Time since diagnosis of diabetes was computed as the age at interview minus the age at fist diagnosis of diabetes. Because the clinical conditions that lead to diabetes and cancer diagnosis may overlap, we required that diabetic participants had been diagnosed of diabetes at least 1 year before the interview. In the same way, to allow for a minimum latency period, all potential confounders that could be modified by the disease (BMI,

tobacco consumption, calorie intake and physical exercise) were censored to one year prior to the interview.

Self-reported diabetic drugs were classified according to the Anatomical Therapeutic Chemical Classification System of the WHO. Because the number of participants per subgroup was small, only two main categories are considered for this report: A10A (insulin and analogues) and A10B (blood glucose lowering drugs, excluding insulin). In accordance to this, diabetic participants have been classified into three different groups regarding the last treatment regimen they had received for at least one year: 1) diabetics under conservative therapy, 2) those treated with blood glucose lowering drugs and 3) diabetics treated with insulin regardless of their use of blood glucose lowering drugs. The duration of each drug use was calculated as the age at the end of treatment (or at the time of interview in the case of current treatments) minus the age at the beginning of treatment.

3.2.3 Tumor classification

Trained personnel reviewed all pathologic records and registered information regarding histological type and receptor status in breast cancer cases, and Gleason score in prostate tumors. Breast cancers were divided into three groups according to the presence/absence of the estrogen receptor (ER), progesterone receptor (PR) or the human epidermal growth factor receptor (HER2) as follows: 1) Hormone receptor positive tumors (ER+ or PR+ with HER2-); 2) HER2+ tumors (independent of ER or PR) and 3) triple negative tumors (ER-, PR- and HER2-). Prostate cancers were classified according to their Gleason score into high- (Gleason >6) and low-grade (Gleason ≤6).

3.2.4 Statistical methods

3.2.4.1 Specific methods for objectives 2.1 and 2.2

Descriptive statistics of participant's characteristics were calculated for both cases and controls. Categorical variables were described using percentages, and continuous variables using means and standard errors. Intra-observer reproducibility of measurements was studied by Pearson's concordance correlation coefficient. The association of the main explanatory variables (age at maximum height; weight at ages 20 and 45; age at maximum

weight; changes in weight since age 20; BMI; WC and WHR ratios) with the risk of breast or prostate tumors, was evaluated using logistic mixed models, including the interviewer as a random effect term, as implemented in Stata´s *gllamm* command. For each explanatory variable, two different regression models were estimated. Model 1 accounted for age, study level, recruitment area and family history of the studied cancer (breast or prostate). Additionally, when breast cancer was the tumor of interest, model 1 adjusted for menopausal status, age of menarche, age at first birth and existence of previous biopsies. Model 2 further adjusted for BMI. In women, both models 1 and 2 were also separately fitted stratified by menopausal status.

Restricted cubic splines with knots at the 10^{th}, 50^{th} and 90^{th} percentiles were used to explore the shape of the dose-response curves for the different variables of interest. These spline models include all the confounders considered in "model 2".

Finally, in order to explore whether the effect of our anthropometric variables differed by cancer subtype, multinomial logistic models were fitted, considering in each case the aforementioned subtypes of breast and prostate cancer, and adjusting again for the confounders included in "model 2". Heterogeneity of effects was tested using a Wald test comparing the slope coefficients obtained for the different cancer subtypes.

To evaluate the consistency of our findings, we performed several sensitivity analyses. First, we repeated all models after excluding those participants whose interviews were not classified as "good" or "excellent" by their interviewers (N=184 for BC analyses; N=38 for PC analyses). Second, we further adjusted the models for diabetic status (yes/no) when this information was available (N_{women}=2949; N_{men}=2178). Third, for breast cancer we repeated the analyses including only those women who had not received hormonal replacement therapy (N=2517). Fourth, when weight gain was the main variable of interest we also adjusted the models for the reported weight at age 20. Finally, we repeated all models stratified by BMI (normoweight/overweight or obese).

3.2.4.2 Specific methods for objectives 3.1 and 3.2

Similarly to what is explained for objectives 2.1 and 2.2, descriptive statistics of participant characteristics were calculated for both cases and controls by diabetes status. To evaluate the association between diabetic status (yes/no) and cancer risk, we fitted multivariate logistic mixed models, including the study region as a random effect term, and

adjusting for age, study level, BMI and family history of the studied cancer. When postmenopausal breast cancer was the outcome, we further adjusted the models for age at menarche, age at first birth and existence of previous biopsies.

To study whether diabetes treatment could be associated with cancer incidence we followed two different strategies. First, we evaluated the risk of cancer associated with different treatment regimens (conservative, oral medication, insulin +/- oral medication) using the non-diabetic population as the reference category. Then we quantified the potential association between duration of use of specific antidiabetic drugs (i.e. metformin, sulfonylurea) and cancer risk in the diabetic population. For both strategies, we used multivariate logistic mixed models with study region as a random effect term, and similar adjustment variables as those described for models on diabetic status.

The association between diabetes duration and cancer risk was assessed again using multivariate logistic mixed models with the same explanatory variables as those previously specified. In these models, diabetes duration was introduced either as a continuous variable, or, in order to evaluate potential nonlinear relationships, as restricted cubic splines with knots at the 10^{th}, 50^{th} and 90^{th} percentiles of its distribution.

Again, we explored if the effects of diabetes, diabetes treatment or diabetes duration could differ by cancer subtype, fitting multinomial logistic models. Heterogeneity of effects was also tested using a Wald test.

As sensitivity analyses we repeated all models after excluding those participants whose interviews has not been classified as “good” or “excellent” by their interviewers. We also adjusted for tobacco consumption, calorie intake and physical exercise when this information was available (N_{women}=1732; N_{men}=2030), and we repeated the models including participants with missing values in BMI (N=177 women and N=47 men) after performing multiple imputation on this variable. We also explored if the effect of diabetes or diabetes treatment varied across categories of BMI by introducing an interaction term between the independent variables and BMI (normoweight/overweight or obese) in the models. Finally, in order to evaluate potential differences in risk associated with frequency of medical visits, we also stratified our results by “screening habits during the last 5 years”.

4 RESULTS

4.1 Results for objective 1

4.1.1 Characteristics of the sample

The mean (± SD) age at study entry was 56 ± 8 years, and 41% of the studied individuals were males. The Strong Heart Study participants had a high prevalence of diabetes (50 %), obesity (50%) and alcohol consumption (42%). The prevalence of cigarette smoking was also high (67% were current or former smokers), although the amount of smoking was relatively low (mean pack-years =16.3).

A total of 225 women and 160 men died from cancer during the studied period. The most common cancer sites were lung and breast in women, and lung and prostate in men. Older participants, participants living in North and South Dakota, participants with lower education, current smokers and never drinkers had higher cancer mortality.

The median (IQR) concentration for the sum of inorganic and methylated arsenic at baseline was 9.7 (5.8-15.6) µg/g creatinine [10.4 (6.12-18.4) µg/L]. Participants living in Arizona and North and South Dakota, participants who had a lower education, current drinkers, and those with lower body mass index and diabetes had higher urine arsenic concentrations (Table 4.1). The median percentage of arsenic species was 8% for inorganic arsenic, 14% for MMA and 78% for DMA.

The median (IQR) concentration of cadmium at baseline was 0.93 (0.61-1.46) µg/g creatinine [1.02 (0.60-1.70) µg/L], with higher levels observed in North and South Dakota (Table 4.2). Urine cadmium concentrations were higher in women, older participants, participants with lower education, current smokers and participants with lower BMI.

Table 4.1 Median (IQR) for the sum of inorganic and methylated arsenic concentrations (µg/g creatinine) by participant characteristics

Variable	Category	N	Median (IQR)	p-value*
Overall		3,935	9.7 (5.8-15.6)	
Age	<55 years	1,947	9.7 (5.9-15.7)	
	55-64	1,292	9.6 (5.7-15.4)	
	>65	696	9.7 (5.6-15.8)	0.28
Sex	Male	1,603	8.8 (5.1-14.3)	
	Female	2,332	10.4 (6.2-16.6)	<0.001
Region	Arizona	1,314	14.3 (9.9-20.8)	
	Oklahoma	1,318	5.6 (3.8-8.2)	
	Dakota	1,303	10.6 (6.9-15.8)	<0.001
Education	No high school	868	13.1 (8.9-20.3)	
	Some high school	992	10.1 (5.9-16.6)	
	Completed high school	2,075	8.0 (5.0-13.2)	<0.001
Smoking	Never	1,284	10.1 (6.0-16.5)	
	Former	1,338	9.2 (5.6-15.0)	
	Current	1,313	9.7 (5.8-15.4)	0.01
Alcohol	Never	636	9.2 (5.5-15.5)	
	Former	1,650	8.7 (5.3-14.4)	
	Current	1,649	10.8 (6.4-17.1)	<0.001
BMI	<25 kg/m^2	610	10.7 (5.8-17.6)	
	25-30	1,324	9.6 (5.6-15.7)	
	>30	2,001	9.5 (5.9-15.0)	0.02
Diabetes	No	1,986	8.4 (5.1-13.6)	
	Yes	1,939	11.0 (6.6-18.0)	<0.001

*p-value from Kruskall-Wallis exact test

Table 4.2 Median (IQR) urine cadmium concentrations (µg/g creatinine) by participant characteristics

Variable	Category	N	Median (IQR)	p-value*
Overall		3792	0.93 (0.61-1.46)	
Age	<55	1883	0.88 (0.57-1.35)	
	55-64	1166	1.00 (0.65-1.56)	
	≥64	743	0.98 (0.63-1.53)	<0.001
Sex	Male	1538	0.71 (0.46-1.08)	
	Female	2254	1.11 (0.74-1.71)	<0.001
Post-menopausal women	Yes	521	1.03 (0.70-1.51)	
	No	1733	1.13 (0.75-1.74)	0.001
Region	Arizona	1268	0.82 (0.55-1.22)	
	Oklahoma	1252	0.87 (0.57-1.35)	
	Dakota	1272	1.13 (0.75-1.80)	<0.001
Education	< High school	834	1.01 (0.66-1.57)	
	High school	965	1.01 (0.65-1.59)	
	>High school	1993	0.88 (0.57-1.34)	<0.001
Smoking status	Never	1284	0.88 (0.57-1.36)	
	Former	1212	0.79 (0.53-1.22)	
	Current	1296	1.14 (0.74-1.73)	<0.001
Cigarette pack-years	0	1284	0.88 (0.57-1.36)	
	1-4	931	0.84 (0.54-1.29)	
	5-19	748	0.93 (0.62-1.44)	
	>= 20	829	1.14 (0.76-1.72)	<0.001
Alcohol	Never	621	1.03 (0.67-1.59)	
	Former	1583	0.91 (0.60-1.46)	
	Current	1588	0.91 (0.59-1.39)	<0.001
BMI, Kg/m^2	<25	591	1.17 (0.75-1.84)	
	25-30	1276	0.96 (0.61-1.50)	
	>=30	1925	0.86 (0.57-1.30)	<0.001

* P-value from Kruskall-Wallis exact test

4.1.2 Association between arsenic exposure and cancer mortality

After multivariate adjustment for age, sex, education, BMI, smoking status and drinking status, the hazard ratio (95% confidence interval) for overall cancer mortality comparing the 80th vs. the 20th percentile of the sum of inorganic and methylated arsenic concentrations in urine was 1.14 (0.92-1.41) (Table 4.3). The corresponding hazard ratios for cancers of the lung, liver, prostate and kidney were 1.56 (1.02-2.39), 1.34 (0.66, 2.72), 3.30 (1.28-8.48) and 0.44 (0.14, 1.14), respectively. For pancreatic cancer, the corresponding hazard ratio was 2.46 (1.09-5.58) and for lymphatic and hematopoietic cancers it was 0.46 (0.22-0.96). Arsenic was not associated with cancers of the esophagus and stomach, colon and rectum, or breast. The linear trend for increased mortality with increasing arsenic levels was statistically significant for lung cancer (p=0.04), prostate cancer (p=0.01) and pancreatic cancer (p=0.03). When modeling the dose-response relationship using restricted cubic splines, we observed no significant departures from linearity (Figure 4.1).

In subgroup analyses by participant characteristics, the fully-adjusted hazard ratio for overall cancer mortality comparing the 80th vs. 20th percentile of arsenic was consistent for most participant subgroups except diabetes at baseline (p for interaction=0.07). No interaction or consistent pattern was observed between urine arsenic concentrations and relative proportions of urine arsenic metabolites in urine (%MMA or %DMA) for overall cancer (Figure 4.2).

In sensitivity analyses adjusting for log-transformed urine creatinine, the hazard ratio for overall cancer mortality comparing the 80th vs. 20th percentile of arsenic was 1.12 (0.88-1.42) and for lung cancer mortality it was 1.53 (0.94-2.48). Among participants without diabetes or albuminuria (N=1,765, 166 overall cancers, 48 lung cancers), the corresponding hazard ratios for overall and lung cancer mortality were 1.29 (0.96-1.73) and 1.35 (0.80-2.27) when adjusting for overall mean specific gravity. Finally, when excluding deaths coded as “malignant neoplasms of other or unspecified sites” (n=26) from the analysis for overall cancer mortality, the results remained similar, and the observed HR (95%CI) comparing the 80th vs 20th percentiles of arsenic distribution was 1.16 (0.93-1.44).

Table 4.3. Hazard ratios (95% CI) for cancer mortality by urine arsenic concentrations

	Sum inorganic and methylated arsenic tertiles			80^{th} vs 20^{th}	
	<6.91 µg/g	6.91-13.32µg/g	>13.32 µg/g	percentiles*	p-trend**
		Overall cancer (ICD-9 140 to 208)			
Cases / Non Cases	121/1,198	135/1,181	130/1,170	386/3,549	
Model 1	1 (Referent)	1.17 (0.90-1.52)	1.27 (0.96-1.70)	1.16 (0.94-1.42)	0.16
Model 2	1 (Referent)	1.17 (0.90-1.53)	1.23 (0.92-1.65)	1.14 (0.92-1.41)	0.24
		Trachea, bronchus, and lung (ICD-9 162)			
Cases / Non Cases	27/1,292	20/1,296	31/1,269	78/3,857	
Model 1	1 (Referent)	0.89 (0.49-1.62)	1.95 (1.09-3.49)	1.59 (1.05-2.42)	0.03
Model 2	1 (Referent)	0.94 (0.51-1.72)	1.82 (1.00-3.32)	1.56 (1.02-2.39)	0.04
		Liver, gallbladder and bile ducts (ICD-9 155-156)			
Cases / Non Cases	8/1311	13/1303	13/1287	34/3901	
Model 1	1 (Referent)	1.51 (0.59-3.88)	1.56 (0.56-4.32)	1.50 (0.76-2.97)	0.24
Model 2	1 (Referent)	1.38 (0.53-3.62)	1.36 (0.47-3.95)	1.34 (0.66-2.72)	0.41
		Prostate (ICD-9 185)			
Cases / Non Cases	6/605	5/526	7/454	18/1,585	
Model 1	1 (Referent)	1.25 (0.37-4.26)	2.90 (0.85-9.92)	1.91 (0.82-4.41)	0.13
Model 2	1 (Referent)	1.55 (0.45-5.33)	4.58 (1.31-16.6)	3.30 (1.28-8.48)	0.01
		Kidney (ICD-9 189.0) †			
Cases / Non Cases	9/1,310	9/1,307	8/1,292	26/3,909	
Model 1	1 (Referent)	0.68 (0.25-1.86)	0.50 (0.15-1.42)	0.69 (0.25-1.90)	0.28
Model 2	1 (Referent)	0.69 (0.25-1.90)	0.44 (0.14-1.40)	0.44 (0.14-1.40)	0.36
		Esophagus and Stomach Cancer (ICD-9 150-151)			
Cases / Non Cases	8/1,311	8/1,308	8/1,292	24/3,911	
Model 1	1 (Referent)	1.19 (0.42-3.39)	1.33 (0.41-4.29)	0.94 (0.40-2.24)	0.89
Model 2	1 (Referent)	1.33 (0.46-3.84)	1.57 (0.47-5.26)	1.09 (0.45-2.66)	0.85
		Pancreas (ICD-9 157)			
Cases / Non Cases	7/1,312	7/1,309	11/1,289	25/3,910	
Model 1	1 (Referent)	1.04 (0.34-3.19)	2.14 (0.67-6.82)	2.26 (1.04-4.88)	0.04
Model 2	1 (Referent)	1.04 (0.33-3.29)	2.14 (0.67-6.82)	2.46 (1.09-5.58)	0.03
		Colon and rectal cancer (ICD-9 153-154)			
Cases / Non Cases	12/1,307	14/1,302	6/1,294	32/3,903	
Model 1	1 (Referent)	1.46 (0.65-3.29)	0.87 (0.30-2.57)	0.83 (0.40-2.24)	0.62
Model 2	1 (Referent)	1.41 (0.62-3.21)	0.82 (0.27-2.48)	0.78 (0.36-1.67)	0.52
		Breast (ICD-9 174) ††			
Cases / Non Cases	7/701	13/772	6/833	26/2,306	
Model 1	1 (Referent)	1.79 (0.67-4.74)	0.90 (0.27-3.07)	0.84 (0.37-1.93)	0.99
Model 2	1 (Referent)	1.92 (0.71-5.15)	0.96 (0.28-3.22)	1.00 (0.44-2.28)	0.69
		Lymphatic and hematopoietic tissue (ICD-9 200–208)			
Cases / Non Cases	11/1,308	20/1,296	9/1,291	40/3,895	
Model 1	1 (Referent)	1.63 (0.74-3.61)	0.69(0.26-1.87)	0.59 (0.29-1.17)	0.13
Model 2	1 (Referent)	1.44 (0.64-3.25)	0.57 (0.21-1.58)	0.46 (0.22-0.96)	0.04

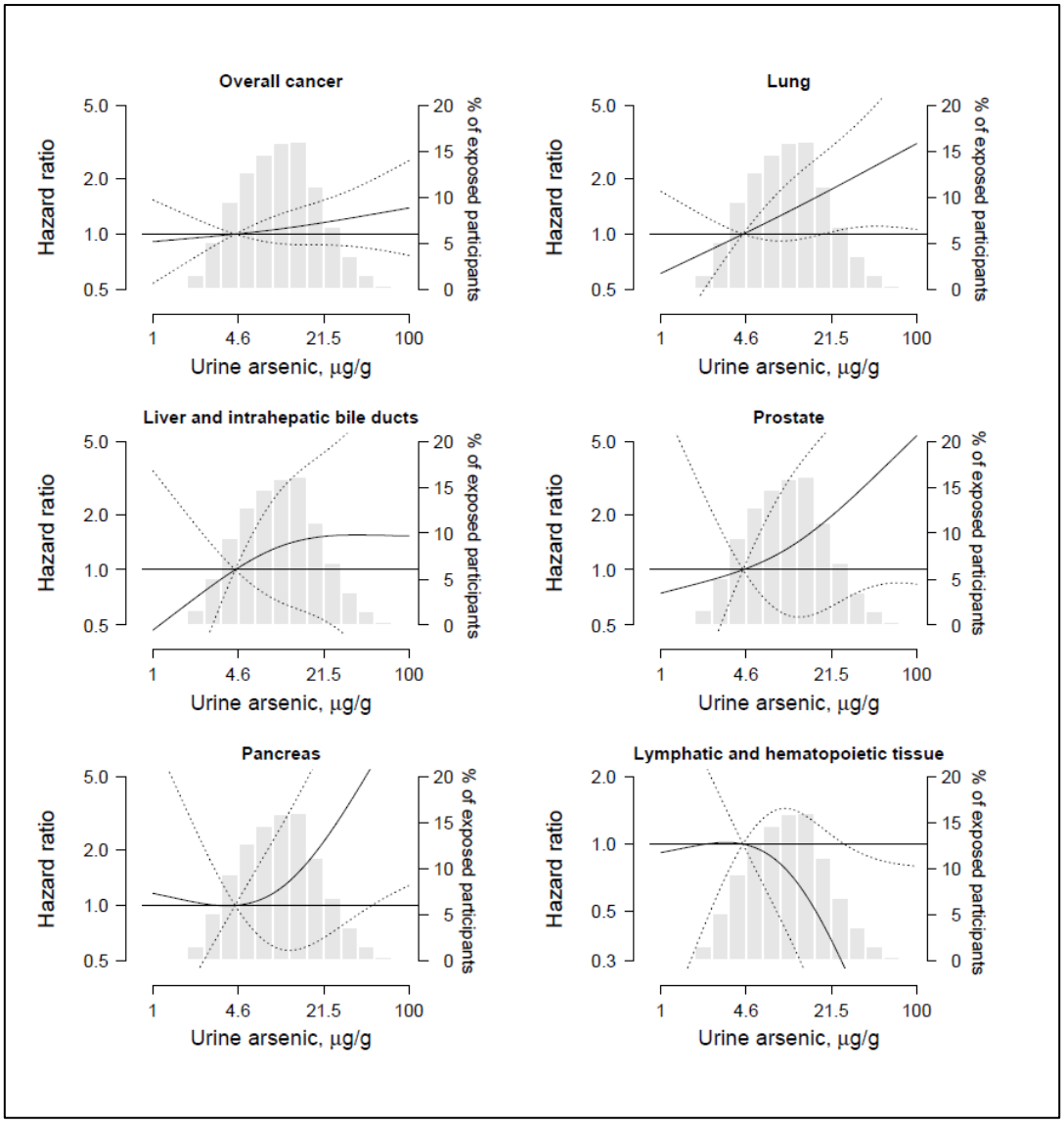
Model 1: Unadjusted, stratified by center. Model 2: Adjusted for, sex, age, education (no high school/some high school/completed high school), smoking status (never, former, current), drinking status (never, former, current), and BMI.

† Model 2 for kidney cancer was further adjusted for estimated glomerular filtration rate (<60, ≥60 ml/min/1.72m^2) and hypertension status (yes/no) †† Model 2 for breast cancer was further adjusted for menopausal status and parity.

*Models comparing the 80^{th} vs 20^{th} percentiles of urine arsenic distributions and associated p-trend were obtained from Cox proportional hazards models with log-transformed arsenic as a continuous variable.

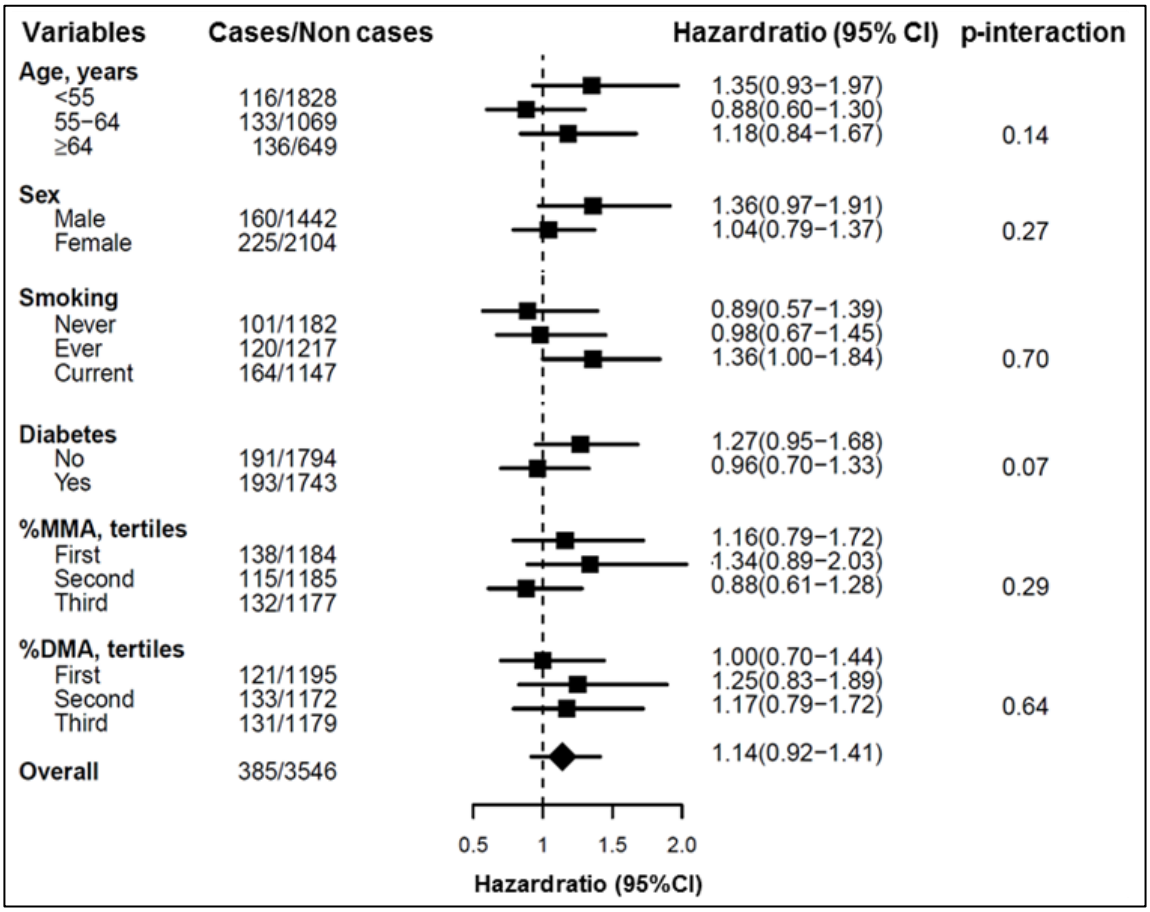
**p trend calculated modeling log-arsenic as continuous

Figure 4.1 Hazard ratios (95%CI) for cancer mortality by urine arsenic concentrations.



Lines represent the hazard ratio (thick line) and 95% confidence intervals (thin line) for overall and specific cancer mortality based on restricted cubic splines for log-transformed sum of inorganic and methylated species with knots at the 10th (3.8 µg/g creatinine), 50th (9.7 µg/g) and 90th (24.0 µg/g) percentiles. The reference was set at the 10th percentile of arsenic distribution. Models were adjusted for age, sex, education (no high school/some high school/completed high school), smoking status (never, former, current), drinking status (never, former, current) and BMI (kg/m^2). Vertical bars represent the histogram of arsenic distribution in the study population.

Figure 4.2 Hazard ratios (95%CI) for cancer mortality comparing the 80th to the 20th percentiles of inorganic arsenic by participant characteristics at baseline.



4.1.3 Association between cadmium exposure and cancer mortality

After multivariate adjustment for age, sex, BMI, smoking status and pack-years (Table 4.4), the hazard ratios (95%CI) for overall and for smoking-related cancer mortality comparing the 80^{th} vs 20^{th} percentile of cadmium concentrations in urine were 1.30 (1.09-1.55) and 1.56 (95%CI: 1.24-1.96), respectively. The corresponding hazard ratios (95%CI) for cancers of the lung and pancreas were 2.27 (1.58-3.27) and 2.40 (1.39-4.17), respectively. After removing current smokers, the hazard ratios (95%CI) for overall, smoking-related, pancreatic and lung cancer mortality were 1.17 (0.93-1.48), 1.37 (1.00-1.87), 2.00 (0.99-2.08) and 2.06 (1.15-3.70). Cadmium was not associated with other cancers. When modeling the dose-response relationship using restricted cubic splines, we found increased risks with increasing urine cadmium concentrations for overall, smoking-related, lung and pancreatic cancer mortality, with no statistically significant departures from linearity (Figure 4.3).

In subgroup analyses, the fully-adjusted hazard ratio for all-cancer mortality and for smoking-related cancer mortality comparing the 80^{th} vs 20^{th} percentiles of cadmium was consistent for all participants' subgroups including smoking status, although the association seemed stronger among current smokers (Figure 4.4).

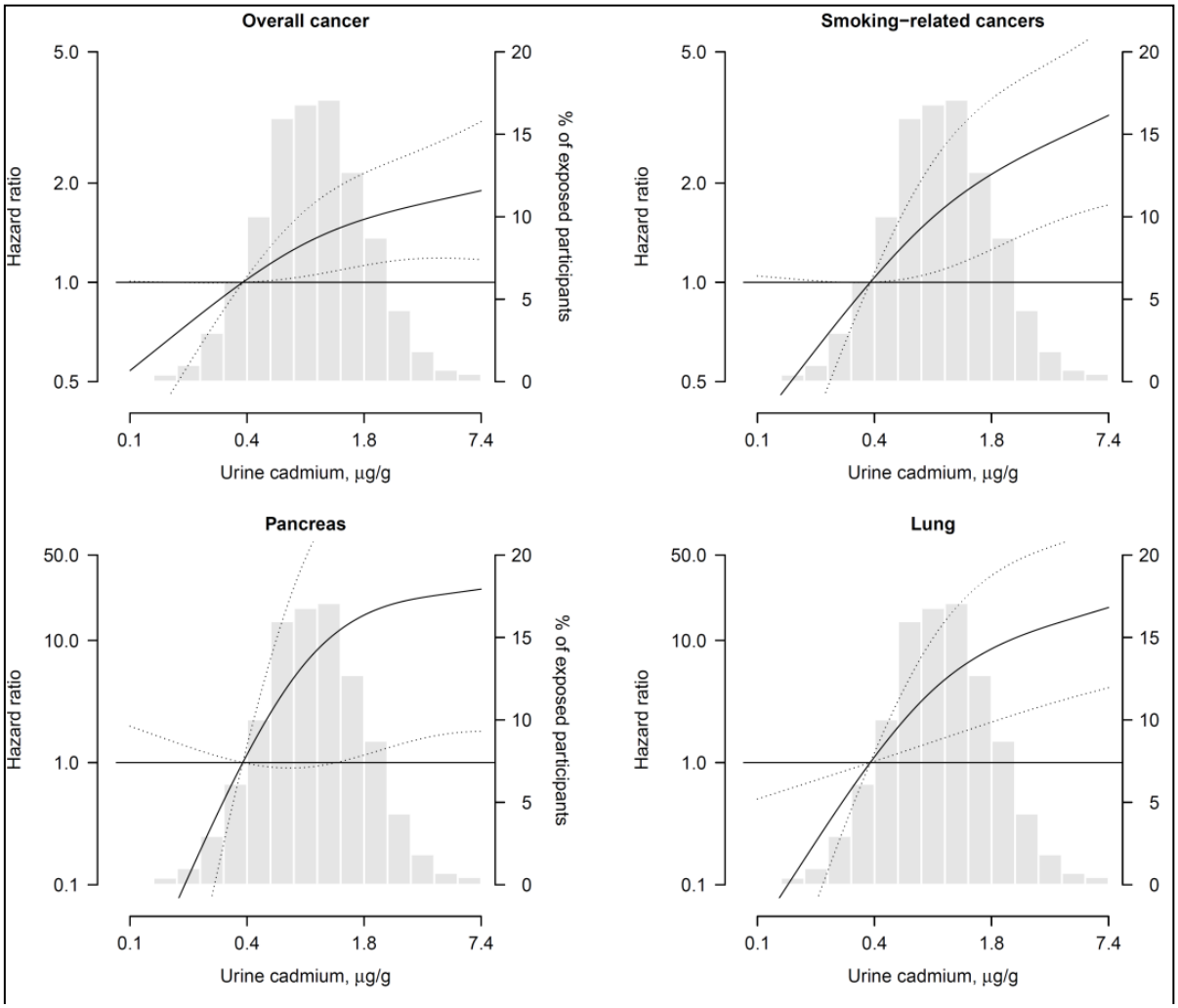
Analyses investigating cadmium as a possible mediator of the association between tobacco smoke and lung cancer mortality showed that the percentage of cancer deaths due to tobacco smoking that could be attributed to cadmium was 9.0% (95%CI: 2.8%-21.8%).

Table 4.4 Hazard ratios (95% CI) for cancer mortality by urine cadmium concentrations

	Cadmium tertiles			80^{th} vs 20^{th}	
	≤0.70	0.71-1.22	≥1.23	percentiles*	p-trend**
Overall cancer (ICD-9 140 to 208)					
Cases / Total	77/1269	142/1266	156/1257	375/3792	
Model 1	1 (Referent)	1.80 (1.36,2.38)	1.94 (1.47,2.57)	1.36 (1.16,1.59)	<0.001
Model 2	1 (Referent)	1.76 (1.32,2.35)	1.85 (1.36,2.51)	1.30 (1.09,1.55)	<0.001
Smoking related cancers* (ICD-9 140-149, 150-151, 153-155, 157, 161, 162, 180, 188-189, 205)**					
Cases / Total	34/1269	72/1266	104/1257	210/3792	
Model 1	1 (Referent)	2.04 (1.36,3.07)	2.81 (1.90,4.16)	1.56 (1.28,1.91)	<0.001
Model 2	1 (Referent)	2.04 (1.34,3.11)	2.80 (1.82,4.31)	1.56 (1.24,1.96)	<0.001
Esophagus and Stomach cancer (ICD-9 150-151)					
Cases / Total	11/1269	6/1266	7/1257	24/3792	
Model 1	1 (Referent)	0.55 (0.20,1.49)	0.68 (0.26,1.79)	0.63 (0.33,1.20)	0.16
Model 2	1 (Referent)	0.60 (0.21,1.68)	0.76 (0.26,2.23)	0.68 (0.34,1.38)	0.29
Colon and rectal cancer (ICD-9 153-154)					
Cases / Total	6/1269	14/1266	12/1257	32/3792	
Model 1	1 (Referent)	2.27 (0.87,5.93)	1.76 (0.65,4.75)	1.06 (0.60,1.86)	0.84
Model 2	1 (Referent)	2.23 (0.82,6.02)	1.74 (0.60,5.11)	0.98 (0.51,1.88)	0.96
Liver and intrahepatic bile ducts (ICD-9 155)					
Cases / Total	4/1269	7/1266	10/1257	21/3792	
Model 1	1 (Referent)	1.79 (0.52,6.14)	2.83 (0.87,9.14)	1.51 (0.81,2.81)	0.20
Model 2	1 (Referent)	2.11 (0.59,7.55)	3.67 (1.01,13.32)	1.64 (0.81,3.13)	0.14
Gallblader and extrahepatic bile ducts (ICD-9 156)					
Cases / Total	3/1269	5/1266	3/1257	11/3792	
Model 1	1 (Referent)	1.56 (0.37,6.57)	0.94 (0.19,4.77)	1.13 (0.44,2.86)	0.80
Model 2	1 (Referent)	1.28 (0.29,5.67)	0.66 (0.11,3.90)	0.89 (0.31,2.54)	0.82
***Pancreas (ICD-9 157)*†**					
Cases / Total	12/1269	-	12/1257	24/3792	
Model 1	1 (Referent)	-	2.00 (0.89,4.52)	2.00 (1.19,3.36)	0.009
Model 2	1 (Referent)	-	2.47 (1.01,6.03)	2.40 (1.39,4.17)	0.002
Bronchus and lung (ICD-9 162)					
Cases / Total	4/1269	21/1266	52/1257	77/3792	
Model 1	1 (Referent)	4.85 (1.66,14.1)	10.2 (3.67,28.4)	2.33 (1.76,3.09)	<0.001
Model 2	1 (Referent)	3.39 (1.14,10.1)	6.65 (2.29,19.3)	2.27 (1.58,3.27)	<0.001
Breast (ICD-9 174)					
Cases / Total	6/504	12/786	7/964	25/1538	
Model 1	1 (Referent)	1.29 (0.48,3.47)	0.60 (0.20,1.83)	1.01 (0.51,1.98)	0.15
Model 2	1 (Referent)	1.34 (1.14,10.1)	0.58 (0.18,1.83)	1.02 (0.50,2.07)	0.96
Prostate (ICD-9 185)					
Cases / *Total*	4/761	8/472	4/289	16/3792	
Model 1	1 (Referent)	1.80 (0.54,6.00)	0.85 (0.2,3.48)	0.70 (0.30,1.62)	0.41
Model 2	1 (Referent)	1.37 (0.40,4.66)	0.48 (0.11,2.08)	0.42 (0.16,1.08)	0.07
Kidney (ICD-9 189)					
Cases / Total	8/1269	11/1266	6/1257	26/3792	
Model 1	1 (Referent)	1.40 (0.56,3.50)	0.82 (0.28,2.42)	0.83 (0.44,1.56)	0.64
Model 2	1 (Referent)	1.92 (0.73,5.01)	1.39 (0.43,4.58)	1.15 (0.58,2.31)	0.61
Lymphohematopoietic tissue (ICD-9 200–208)					
Cases / Total	6/1269	17/1266	14/1257	37/3792	
Model 1	1 (Referent)	2.96 (1.16,7.52)	2.73 (1.04,7.20)	1.45 (0.87,2.40)	0.15
Model 2	1 (Referent)	2.94 (1.12,7.70)	2.79 (0.99,7.90)	1.40 (0.80,2.43)	0.24

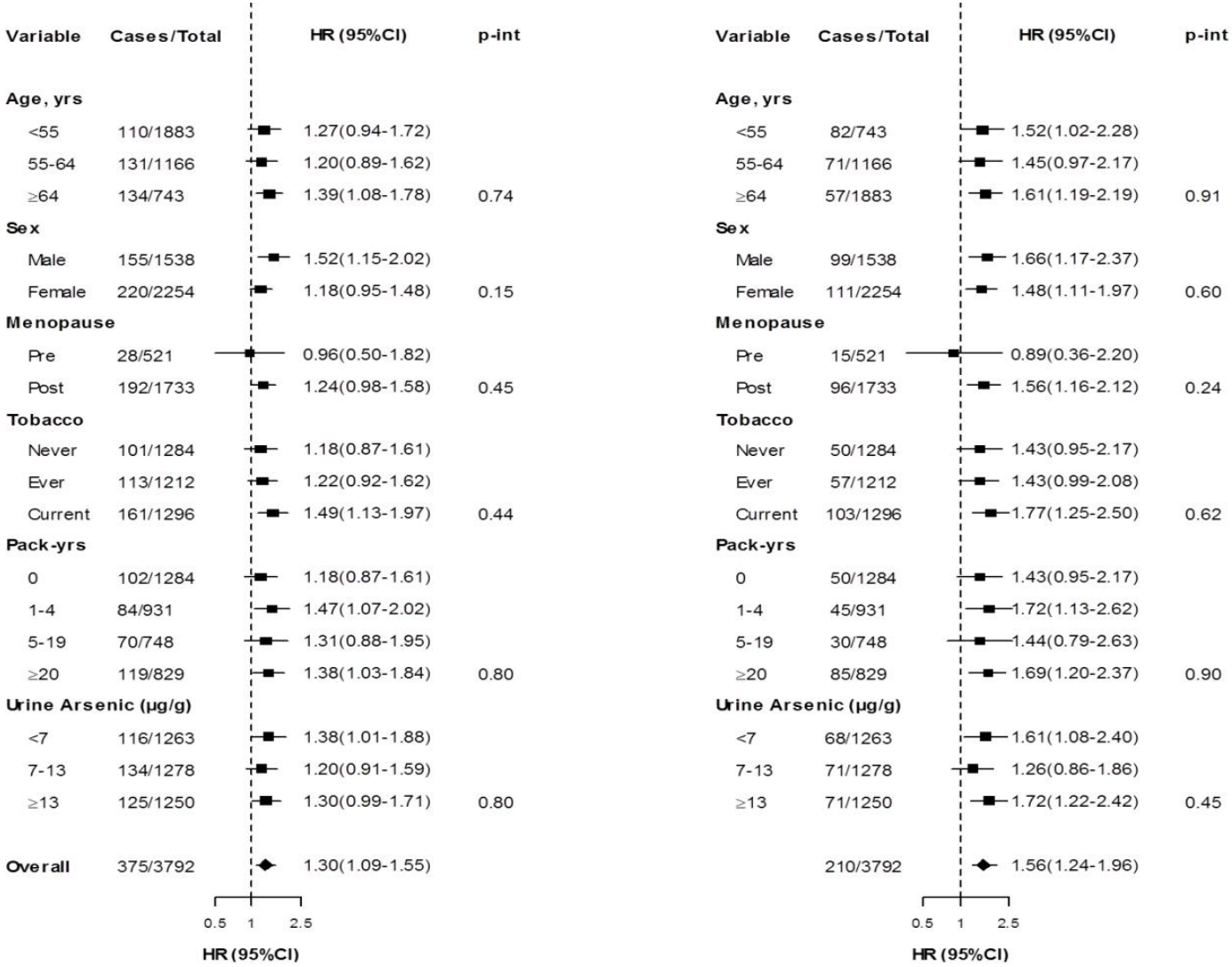
Model 1: Unadjusted, stratified by center. Model 2: Adjusted for sex, age, smoking status (never, former, current),number of packs-per year and BMI (kg/m^2). Model 2 for breast cancer was further adjusted for menopausal status, parity and hormonal replacement use. Model 2 for kidney cancer was further adjusted for estimated glomerular filtration rate (continuous) and hypertension status (yes,no). † Tertiles 1 and 2 are combined because there was only one case in the first tertile. *Models comparing the 80^{th} vs 20^{th} percentiles of urine cadmium and associated p-trend were obtained from Cox proportional hazards models with log-transformed cadmium as a continuous variable. **p-trend calculated modeling log-cadmium as continuous. *** Smoking related cancers: Lip, oral cavity and pharynx (140-149), esophagus (150), stomach (151), colon and rectum (153-154), liver (155), pancreas (157), larynx (161), trachea, bronchus and lung (162), cervix (180), bladder (188), kidney (189), myeloid leukemia (205).

Figure 4.3 Hazard ratios (95%CI) for cancer mortality by urine cadmium concentrations



Hazard ratios (95% confidence intervals) for overall, smoking-related, lung and pancreas cancer mortality based on restricted cubic splines for log-transformed urine cadmium concentrations with knots at the 10th, 50th and 90th percentile. The reference value is set at the 10th percentile of the cadmium distribution. Hazard ratios are adjusted for sex, age, smoking status, pack-years and BMI.
Vertical bars represent the histogram of urine cadmium distribution

Figure 4.4 Hazard ratios (95%CI) for cancer mortality comparing the 80th to the 20th percentiles of cadmium by participant characteristics at baseline.



4.2 Results for objective 2

4.2.1 Characteristics of the sample

Table 4.5 shows the main socio-demographic characteristics and anthropometric measurements among breast cancer cases and controls by menopausal status, and among prostate cancer cases and controls. Both pre-and postmenopausal cases had lower education levels, while postmenopausal cases were slightly younger than their controls. Although the percentage of obese women in premenopausal cases was lower than in controls, postmenopausal cases were more likely to have higher self-reported values of weight at age 45 and BMI 1 year prior to the interview, as well as greater WC and WHR at the time of the interview. On average, prostate cancer cases reached their maximum weight one year later, weighted less at age 45, and were shorter than their controls. Additionally, prostate cancer cases presented a larger WHR at the time of the interview.

Cases were interviewed within 60 (BC) or 80 (PC) days from their diagnosis. Interviewers classified participant responses as follows: fair (6% for breast and 2% for prostate participants), good (46% for breast and 37% for prostate) or excellent (48% for breast and 59% for prostate). Results from the Pearson´s correlation test indicated a high intra-observer reproducibility of measurements (r=0.99 for all of them).

The correlation coefficients between the different anthropometric variables can be found in table 4.6. Variables highly correlated ($r \geq 0.60$) with the "BMI 1 year prior to interview" included: "weight gain since age 20", "weight at age 45" and "waist circumference at the time of the interview".

Table 4.5: MCC-Spain: Socio-demographic characteristics and anthropometric measurements among breast cancer cases and controls by menopausal status, and among prostate cancer cases and controls.

Variable	Premenopausal women			Postmenopausal somwn			Men		
	Cases (N=623)	Controls (N=537)	p-val	Cases (N=864)	Controls (N=963)	p-val	Cases (N=1018)	Controls (N=1327)	p-val
Socio-demographic factors									
Age (mean, SD)	45.1 (7.8)	45.5 (9.4)	0.36	63.3 (8.9)	64.5 (9.5)	**<0.01**	66.0 (7.24)	66.6 (8.28)	0.05
Study level, N (%)									
< Primary	17 (3)	22 (4)		194 (22)	194 (21)		235 (23)	257 (19)	
Primary	147 (24)	106 (20)		337 (29)	341 (35)		411 (41)	436 (33)	
High School	270 (43)	211 (39)		223 (26)	270 (28)		214 (21)	361 (27)	
>High School	189 (30)	198 (37)	**0.04**	110 (13)	158 (16)	**0.05**	158 (15)	273 (21)	**<0.01**
Reproductive factors									
Age of menarche (mean, SD)	12.7 (1.4)	12.7 (1.5)	0.32	12.9 (1.6)	12.9 (1.7)	0.95	-	-	-
Age at first birth age (mean, SD)	27.8 (0.7)	27.7 (0.7)		26.2 (4.5)	26.5 (4.5)	0.24	-	-	-
Anthropometric variables*									
Age reached maximum height (mean, SD)	15.9 (2.4)	16.1 (2.6)	0.18	15.8 (2.5)	16.3 (2.9)	**<0.01**	19.5 (2.7)	19.4 (2.7)	0.92
Age reached maximum weight (mean, SD)	39.7 (11.0)	39.0(0.49)	0.36	54.7 (0.5)	54.6 (14.9)	0.90	58.0 (13.6)	57.1 (14.2)	0.15
Weight at age 20 (mean, SD)	54.7 (7.7)	55.3 (9.2)	0.22	54.0 (9.3)	53.8 (8.6)	0.77	65.4 (9.5)	65.9 (9.7)	0.35
Weight at age 45 (mean, SD)	63.3 (11.8)	62.0(11.9)	0.17	63.2(12.5)	61.0 (11.3)	**<0.01**	73.1 (11.5)	74.2 (11.2)	**0.03**
Weight gain since age 20 (mean, SD)	8.7 (10.0)	8.4(10.3)	0.58	14.5(12.1)	12.5 (11.3)		13.7 (11.6)	13.9 (12.2)	0.82
Height meters (1-yr before diagnosis)	1.62 (0.06)	1.62 (0.06)	0.21	1.58 (0.1)	1.59 (0.06)	0.33	1.69 (0.07)	1.70 (0.07)	**0.01**
BMI (1-yr before interview), N (%)									
20-24	402 (64)	345 (64)		297 (34)	426 (44)		254 (25)	328 (25)	
25-29	167 (27)	125 (23)		346 (40)	341 (35)		529 (52)	691 (52)	
>29	54 (9)	67 (13)	0.07	221 (26)	196 (21)	**<0.01**	235 (23)	308 (23)	0.99
Waist Ø (tertiles), N (%)									
Women <82; Men <98 cm	268 (45)	272 (51)		140 (17)	215 (22)		300 (33)	424 (32)	
Women 82-93.5; Men 98-106 cm	193 (33)	156 (29)		269 (34)	340 (36)		316 (25)	442 (34)	
Women: ≥93.5; Men ≥106 cm	132 (22)	104 (20)	0.14	392 (49)	396 (42)	**<0.01**	295 (32)	447 (34)	0.71
Waist-to-hip ratio (tertiles), N (%)									
Women: <0.81; Men <0.94 cm	285 (48)	279 (53)		172 (22)	233 (25)		248 (28)	436 (33)	
Women 0.81-0.88; Men 0.94-0.99 cm	184 (31)	155 (29)		256 (32)	336 (35)		302 (33)	435 (33)	
Women ≥0.88; Men ≥0.99 cm	124 (21)	98 (18)	0.32	371 (46)	382 (40)	**0.03**	356 (39)	441 (33)	**<0.05**
Family history of breast cancer									
None	507 (81)	488 (91)		730 (84)	855 (89)		847 (83)	1249 (94)	
1 first degree	83 (13)	37 (7)		106 (2)	87 (9)		8 (1)	3 (0.2)	
>1 first degree	12 (2)	0 (0)		15 (12)	10 (1)		137 (13)	72 (5.6)	
≥1 second degree	21 (4)	12 (2)	**<0.01**	13 (2)	11 (1)	0.06	26 (3)	3 (0.2)	**<0.05**
Previous biopsies									
No	571 (92)	521 (97)		768 (89)	949 (99)		-	-	-
Yes	52 (8)	16 (3)	**<0.01**	96 (11)	14 (1)	**<0.01**	-	-	-

Data in the table are percentages for categorical variables or means ± SD for continuous variables.

Table 4.6: MCC-Spain: Correlation coefficients between the studied anthropometric variables by sex

	Age max height	Age max weight	Weight age 20	Weight age 45	Weight gain	BMI	Waist circumference	Waist-hip ratio
Women								
Age max height	1.00							
Age max weight	0.06*	1.00						
Weight age 20	0.07*	-0.17*						
Weight age 45	0.06*	0.00	0.54*	1.00				
Weight gain	0.07*	0.44*	-0.27*	0.35*				
BMI	0.07*	0.36*	0.32*	0.60*	0.74*	1.00		
Waist Ø	0.05*	0.39*	0.27*	0.51*	0.62*	0.76*	1.00	
Waist-hip ratio	-0.01	0.27*	0.02	0.15*	0.29*	0.34*	0.63*	1.00
Men								
Age max height	1.00							
Age max weight	0.04	1.00						
Weight age 20	-0.00	-0.23*	1.00					
Weight age 45	0.00	-0.18*	0.64*	1.00				
Weight gain	0.01	0.36*	-0.36*	0.14	1.00			
BMI	-0.00	0.23*	0.21*	0.43*	0.71*	1.00		
Waist Ø	0.05*	0.26*	0.22*	0.39*	0.58*	0.73*	1.00	
Waist-hip ratio	0.12*	0.16*	-0.02	0.06*	0.25*	0.29*	0.49*	1.00

*p <0.05 according to Spearman´s correlation test

4.2.2 Association between obesity and other related anthropometric factors and breast cancer risk.

Table 4.7 presents the ORs (95%CI) for the associations between the studied anthropometric variables and breast cancer risk. The age at maximum height was inversely associated with breast cancer risk ($OR_{\text{per year}}$=0.95: 95%CI=0.95-0.99). An overall increased risk of breast cancer was observed with increasing weight at age 45 ($OR_{5\ kg}$=1.05: 95%CI 1.00-1.11), increasing BMI ($OR_{2\ units}$=1.05: 95%CI 1.01-1.09) or WHR ($OR_{0.10\ units}$=1.11: 95%CI 0.99-1.26). Although a positive link was also observed for weight gain since age 20, this association disappeared when final BMI was taken into account. The right columns of table 4.7 present the analysis in pre- and postmenopausal women, and figure 4.5 shows the dose-response shape of the

association between BMI and WHR in both groups of women. Stratified analyses by menopausal status revealed that the effect of BMI and the WHR was mainly restricted to postmenopausal women (see also figure 4.5). Interestingly, these analyses also indicate that the association of breast cancer with WC in premenopausal women was only present after accounting for BMI, while in postmenopausal women this observed risk was independent of BMI.

Table 4.8 summarizes the results for breast cancer models by tumor subtypes. Similar associations with BMI, WC, WHR, weight at age 45 and age at maximum height were found for HR+/HER2-. HER2+ and TN cancers, both in pre-and postmenopausal women. Those variables associated to obesity earlier in like ("weight at age 20" and "age at maximum weight) were associated with a decreased risk of more aggressive tumor subtypes (HER2+ and triple negative) in premenopausal but not postmenopausal women. In postmenopausal women, significant heterogeneity of the ORs across intrinsic subtypes could be observed for "age at maximum weight" and for "weight gain since age 20". Interestingly, in models that did not account for BMI, a positive association was seen between "weight gain since age 20" and the risk of HR+/HER2- tumors in postmenopausal women (OR=1.10; 95%CI:1.04.1.16); data not shown in tables.

In sensitivity analyses, results were similar when excluding interviews classified as "fair" (data not shown), as well as when analyses were further adjusted for diabetes status or stratified by BMI. Consistent findings were also found after exclusion of women who had received hormonal replacement therapy, although the ORs for all these variables in postmenopausal women were slightly higher (see table 4.9).

Table 4.7: MCC-Spain: Odds ratios (95%CI) for the association between anthropometrical variables and breast cancer risk, overall and by menopausal status

	OVERALL						PREMENOPAUSAL						POSTMENOPAUSAL						p-het
	N		Model 1		Model 2		N		Model 1		Model 2		N		Model 1		Model 2		
	Co	Ca	OR (95%CI)	p-trend	OR (95%CI)	p-trend	Co	Ca	OR (95%CI)	p-trend	OR (95%CI)	p-trend	Co	Ca	OR (95%CI)	p-trend	OR (95%CI)	p-trend	
Age maximum height (per year)	1055	1089	**0.95 (0.91- 0.99)**	**0.02**	**0.95 (0.91-0.99)**	**<0.01**	428	479	0.99 (0.91-1.03)	0.35	0.97 (0.91-1.04)	0.41	627	610	**0.95 (0.90-0.99)**	**0.03**	**0.93 (0.89-0.98)**	**0.01**	0.84
Weight at age 20 (per 5 kg)	1382	1343	1.03 (0.98-1.08)	0.31	1.00 (0.95-1.06)	0.87	510	583	1.02 (0.94-1.11)	0.63	1.03 (0.96-1.15)	0.27	872	760	1.03 (0.96-1.09)	0.46	0.98 (0.92-1.05)	0.60	0.79
Weight at age 45 (per 5 kg)	1163	1124	**1.08 (1.03-1.12)**	**<0.01**	**1.05 (1.00-1.11)**	**0.07**	285	344	1.05 (0.97-1.14)	0.24	**1.13 (1.00-1.28)**	**0.05**	878	780	**1.09 (1.03-1.14)**	**<0.01**	1.04 (0.98-1.10)	0.22	0.46
Age maximum weight (5 years)	1445	1435	1.02 (0.99-1.06)	0.22	1.01 (0.97-1.05)	0.61	528	600	1.04 (0.96-1.13)	0.30	1.06 (0.97-1.14)	0.19	917	835	1.02 (0.97-1.06)	0.47	0.99 (0.95-1.03)	0.67	0.11
Weight gain since age 20 (per 5 kg)	1339	1312	**1.04 (1.01-1.08)**	**0.05**	1.00 (0.95-1.07)	0.90	477	555	0.97 (0.90-1.04)	0.40	1.00 (0.90-1.12)	1.00	862	757	**1.08 (1.04-1.14)**	**<0.01**	1.01 (0.94-1.09)	0.79	0.45
BMI																			
20-24	771	699	**1.00**		-		345	402	1.00		-		426	297	1.00		-		
25-29	466	513	**1.32 (1.09-1.60)**		-		125	167	1.22 (0.88-1.67)		-		341	346	**1.49 (1.17-1.91)**		-		
>29	263	275	**1.31 (1.03-1.66)**	**<0.01**	-		67	54	0.66 (0.41-1.06)	0.45	-		196	221	**1.71 (1.28-2.28)**	**<0.01**	-		
Per 2 units BMI	1500	1487	**1.05 (1.01-1.09)**	**<0.01**	-	-	537	623	0.97 (0.90-1.03)	0.28	-	-	963	864	**1.10 (1.05-1.15)**	**<0.01**	-	-	**0.01**
Waist tertiles																			
<81.4 cm	487	408	**1.00**		**1.00**		272	268	1.00		**1.00**		215	140	**1.00**		**1.00**		
81.4-93.0 cm	496	462	**1.27 (1.03-1.58)**		**1.24 (0.99-1.56)**		156	193	1.20 (0.88-1.65)		**1.42 (1.00-2.01)**		340	269	**1.41 (1.04-1.93)**		**1.31 (0.95-1.80)**		
≥93.0 cm	500	524	**1.52 (1.20-1.93)**	**<0.01**	**1.44 (1.07-1.95)**	**0.02**	104	132	1.19 (0.81-1.75)	0.27	**1.83 (1.08-3.11)**	**0.02**	396	392	**1.90 (1.04-1.93)**	**<0.01**	**1.54 (1.04-2.28)**	**0.03**	
Per 10 cm waist	1483	1394	**1.12 (1.04-1.20)**	**<0.01**	1.06 (0.95-1.18)	0.33	532	593	1.02 (0.91-1.15)	0.71	1.18 (0.98-1.42)	0.08	951	801	**1.17 (1.06-1.29)**	**<0.01**	1.04 (0.99-1.20)	0.08	0.56
WHR tertiles																			
<0.81	537	465	**1.00**		**1.00**		279	285	1.00		1.00		233	172	**1.00**		**1.00**		
0.81-0.88	532	463	**1.22 (0.99-1.51)**		**1.18 (0.95-1.47)**		155	184	1.30 (0.94-1.79)		1.39 (1.00-1.93)		336	256	**1.20 (0.89-1.61)**		**1.13 (0.83-1.52)**		
≥0.88	524	537	**1.40 (1.11-1.77)**	**<0.01**	**1.32 (1.03-1.68)**	**0.03**	98	124	1.22 (0.83-1.80)	0.20	1.38 (0.91-2.09)	0.09	382	371	**1.57 (1.15-2.13)**	**<0.01**	**1.39 (1.01-1.91)**	**0.04**	
Per 0.1 units WHR	1483	1392	**1.15 (1.02-1.30)**	**<0.01**	1.11 (0.99-1.26)	0.08	532	593	1.04 (0.86-1.27)	0.69	1.10 (0.89-1.36)	0.39	951	799	**1.28 (1.09-1.49)**	**<0.01**	**1.20 (1.03-1.41)**	**0.02**	0.14

Model 1: Adjusted for age, study level (<Primary, Primary, High school, >High school), age at first birth (25-29, <20, 20-24,>29, nulliparous), age at menarche (12-13, <12, >13), previous biopsies (yes/no), family history of breast cancer. Models for the overall population are further adjusted for menopausal status.

Model 2: Further adjusted for BMI (1-yr before diagnosis).

p-het: p for heterogeneity of effects by menopausal status as estimated in model 2.

Table 4.8: MCC-Spain: Odds ratios (95%CI) for the association between the studied anthropometrical variables and breast cancer risk by menopausal status and histological subtype.

	PREMENOPAUSAL											POSTMENOPAUSAL									
	CO	HR+/HER2-			HER2+			TN				CO	HR+/HER2-			HER2+			TN		
	N	N	OR (95%CI)	p-trend	N	OR (95%CI)	p-trend	N	OR (95%CI)	p-trend	p-het*	N	N	OR (95%CI)	p-trend	N	OR (95%CI)	p-trend	N	OR (95%CI)	p-trend	p-het*
Age maximum height (per year)	428	307	0.98 (0.91-1.05)	0.62	73	0.97 (0.86-1.09)	0.56	44	0.99 (0.86-1.13)	0.87	0.97	627	387	0.95 (0.90-1.01)	0.08	92	**0.89 (0.80-0.98)**	**0.02**	56	0.93 (0.83-1.05)	0.27	0.44
Weight at age 20 (per 5 kg)	510	355	1.01 (0.90-1.14)		85	**0.82 (0.71-0.95)**	**0.03**	38	0.88 (0.72-1.08)		0.85	872	483	0.97 (0.90-1.05)	0.51	107	**1.09 (0.97-1.22)**	0.13	75	1.04 (0.91-1.19)	0.57	0.12
Weight at age 45 (per 5 kg)*	285	244	**1.07 (1.02-1.13)**	**<0.01**	36	1.04 (0.95.1.13)	0.43	25	**1.13 (1.04-1.24)**	<0.01	0.32	878	498	1.03 (0.96-1.10)	0.40	111	1.00 (0.90-1.12)	0.99	82	1.10 (0.99-1.23)	0.08	0.35
Age maximum weight (per 5 years)	528	397	1.04 (0.95-1.12)	0.45	87	1.13 (0.96-1.33)	0.19	51	1.24 (0.98-1.56)	0.07	0.20	917	529	1.00 (0.96-1.05)	0.88	121	1.05 (0.96-1.15)	0.29	85	**0.89 (0.83-0.97)**	**<0.01**	**<0.01**
Weight gain since age 20** (per 5 kg)	477	369	1.05 (0.94-1.17)	0.37	80	1.08 (0.92-1.30)	0.39	48	1.18 (0.92-1.51)	0.20	0.72	862	459	1.03 (0.95-1.12)	0.47	108	0.89 (0.79-1.01)	0.07	66	0.94 (0.82-1.08)	0.41	**0.05**
BMI						-				-												
20-24	345	259	1.00		58	1.00		35	1.00			426	190	1.00		44	1.00		28	1.00		
25-29	125	121	**1.42 (1.00-2.01)**		19	1.00 (0.55-1.81)		13	1.14 (0.56-2.30)			341	219	**1.46 (1.11-1.92)**		54	**1.73 (1.10-2.72)**		30	**1.30 (0.73-2.24)**		
>29	67	33	0.63 (0.37-1.08)	0.75	14	1.32 (0.64-2.73)	0.47	4	0.62 (0.20-2.30)	0.63		196	139	**1.64 (1.19-2.26)**	**<0.01**	27	**1.54 (0.89-2.66)**	**0.06**	30	**2.31 (1.30-4.11)**	**<0.01**	
Per 2 units BMI	537	413	0.97 (0.91-1.05)	0.48	91	1.05 (0.94-1.17)	0.41	52	0.93 (0.80-1.09)	0.38	0.34	963	548	**1.10 (1.05-1.16)**	**<0.01**	125	**1.09 (1.00-1.19)**	**0.05**	88	1.10 (0.99-1.21)	0.06	0.97
Waist tertiles																						
<81.4 cm	272	171	**1.00**		41	1.00		20	1.00			215	88	1.00		20	1.00		13	1.00		
81.4-93.0 cm	156	133	**1.55 (1.06-2.26)**		27	1.00 (0.55-1.84)		16	1.93 (0.88-4.24)			340	176	1.29 (0.90-1.86)		32	1.26 (0.66-2.38)		22	1.06 (0.50-2.27)		
≥93.0 cm	104	93	**2.19 (1.22-3.90)**	**<0.01**	19	1.11 (0.45-2.73)	0.84	9	2.42 (0.74-7.85)	0.09		396	243	1.39 (0.90-2.15)	0.17	65	**2.45 (1.22-4.93)**	**<0.01**	44	1.78 (0.78-4.05)	0.11	
Per 10 cm waist	532	397	**1.21 (0.99-1.49)**	0.06	87	1.02 (0.75-1.40)	0.88	45	1.40 (0.96-2.06)	0.08	0.40	951	507	1.02 (0.87-1.19)	0.79	117	1.19 (0.94-1.51)	0.15	79	1.05 (0.80-1.39)	0.72	0.42
WHR tertiles																						
<0.81	279	156	1.00		37	1.00		15	1.00			233	106	1.00		21	1.00		19	1.00		
0.81-0.88	155	117	1.1 (0.82-1.57)		25	1.21 (0.69-2.12)		14	0.69 (0.39-1.26)			336	170	1.15 (0.82-1.62)		37	1.34 (0.74-2.43)		20	0.68 (0.34-1.34)		
≥0.88	98	81	1.20 (0.84-1.71)	0.15	17	**1.83 (1.03-3.26)**	**0.08**	6	1.07 (0.59-1.93)	0.06		382	230	1.30 (0.91-1.86)	0.15	58	**1.94 (1.07-3.51)**	**0.02**	40	1.14 (0.60-2.17)	0.46	
Per 0.1 units WHR	532	397	1.08 (0.85-1.36)	0.52	87	1.12 (0.77-1.62)	0.58	45	1.33 (0.84-2.13)	0.23	0.66	951	506	1.17 (0.99-1.39)	0.09	116	**1.35 (1.02-1.80)**	**0.04**	79	1.12 (0.81-1.56)	0.51	0.55

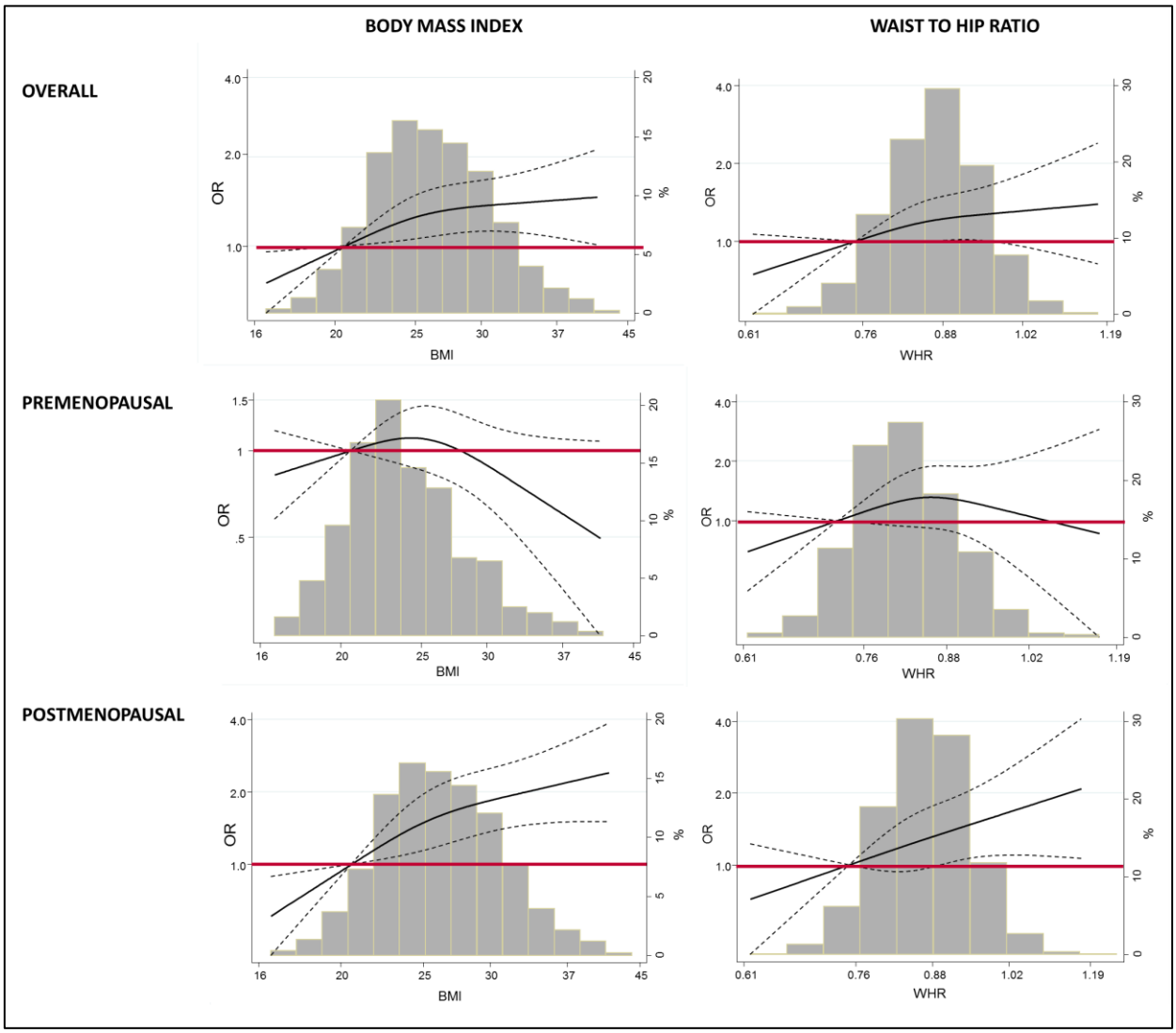
Models are adjusted for age, study level (<Primary, Primary, High school, >High school), recruitment area, BMI 1-year before the interview (continuous) age at first birth (25-29, <20, 20-24,>29, nulliparous), age at menarche (12-13, <12, >13), previous biopsies (yes/no), family history of breast cancer. Co:Control; p-het: p for heterogeneity among intrinsic subtypes.

Table 4.9: MCC-Spain: Odds Ratios (95%CI) for the association between anthropometrical variables and breast cancer risk among non HRT users, overall and by menopausal status

	OVERALL						PREMENOPAUSAL						POSTMENOPAUSAL					
	N		Model 1		Model 2		N		Model 1		Model 2		N		Model 1		Model 2	
	Co	Ca	OR (95%CI)	p-trend	OR (95%CI)	p-trend	Co	Ca	OR (95%CI)	p-trend	OR (95%CI)	p-trend	Co	Ca	OR (95%CI)	p-trend	OR (95%CI)	p-trend
Age maximum height **(per year)**	890	965	**0.96 (0.92-1.00)**	**0.06**	**0.96 (0.91-0.99)**	**0.04**	402	468	0.96 (0.90-1.03)	0.26	0.96 (0.90-1.03)	0.30	488	497	0.96 (0.91-1.02)	0.24	**0.95 (0.89-1.00)**	**0.10**
Weight at age 20 **(per 5 kg)**	1122	1188	1.04 (0.99-1.10)	0.14	1.02 (0.96-1.09)	0.45	473	569	1.01 (0.93-1.11)	0.74	1.05 (0.95-1.15)	0.36	649	619	1.06 (0.99-1.14)	0.11	1.01 (0.94-1.10)	0.74
Weight at age 45 **(per 5 kg)**	915	965	**1.09 (1.04-1.15)**	**<0.01**	**1.08 (1.02-1.14)**	**0.01**	259	329	1.07 (0.98-1.16)	0.15	**1.14 (1.00-1.31)**	**0.05**	656	636	**1.11 (1.05-1.18)**	**<0.01**	1.07 (1.00-1.15)	**0.04**
Age maximum weight **(per 5 years)**	1172	1262	1.03 (0.99-1.07)	0.19	1.02 (0.97-1.06)	0.46	487	584	1.04 (0.96-1.13)	0.29	1.06 (0.97-1.15)	0.19	685	678	1.02 (0.97-1.07)	0.38	0.99 (0.95-1.05)	0.83
Weight gain since age 20 **(per 5 kg)**	1088	1159	**1.04 (1.00-1.08)**	0.09	0.99 (0.93-1.06)	0.87	442	541	0.98 (0.90-1.05)	0.52	1.01 (0.90-1.13)	0.97	646	618	**1.08 (1.02-1.14)**	**<0.01**	0.99 (0.91-1.07)	**<0.01**
BMI																		
20-24	637	637	**1.00**		-		320	392	1.00		-		317	245	1.00		-	
25-29	363	434	**1.28 (1.04-1.58)**		-		116	161	1.19 (0.85-1.67)		-		247	273	**1.50 (1.12-1.99)**		-	
>29	209	237	**1.29 (1.00-1.68)**	**0.03**	-		59	53	0.68 (0.41-1.13)		-		150	184	**1.77 (1.27-2.45)**	**<0.01**	-	
Per 2 units BMI	1209	1308	**1.05 (1.00-1.09)**	**0.03**	-		495	606	0.97 (0.90-1.04)	0.36	-		714	702	**1.10 (1.05-1.17)**	**<0.01**	-	
Waist tertiles																		
<81.4 cm	434	407	**1.00**		**1.00**		252	260	1.00		1.00		158	103	**1.00**		**1.00**	
81.4-93.0 cm	398	380	**1.25 (1.00-1.58)**		**1.25 (0.98-1.60)**		144	189	1.22 (0.88-1.70)		1.39 (0.97-2.00)		259	216	**1.44 (1.00-2.06)**		**1.35 (0.93-1.97)**	
≥93.0 cm	363	439	**1.53 (1.18-1.98)**	**<0.01**	**1.52 (1.09-2.14)**	**<0.01**	96	128	1.10 (0.73-1.64)	0.47	1.54 (0.88-2.71)	0.08	286	332	**2.14 (1.48-3.09)**	**<0.01**	**1.80 (1.14-2.85)**	**0.01**
Per 10 cm waist	1195	1226	**1.11 (1.02-1.20)**	**0.01**	1.10 (0.97-1.24)	0.13	492	577	1.01 (0.87-1.14)	0.87	1.13 (0.93-1.38)	0.21	703	649	**1.23 (1.10-1.37)**	**<0.01**	1.14 (0.97-1.35)	0.11
WHR tertiles																		
<0.81	410	363	**1.00**		**1.00**		264	280	1.00		1.00		170	127	**1.00**		**1.00**	
0.81-0.88	403	405	**1.26 (1.00-1.58)**		1.23 (0.97-1.55)		142	176	1.26 (0.90-1.76)		1.35 (0.95-1.90)		256	204	**1.30 (0.92-1.85)**		**1.22 (0.86-1.74)**	
≥0.88	382	460	**1.52 (1.18-1.97)**	**<0.01**	**1.46 (1.12-1.91)**	**<0.01**	86	121	1.25 (0.83-1.89)	0.18	1.41 (0.90-2.19)	0.08	277	318	**1.84 (1.29-2.64)**	**<0.01**	**1.64 (1.14-2.38)**	**<0.01**
Per 0.1 units WHR	1195	1228	**1.24 (1.08-1.41)**	**<0.01**	**1.21 (1.05-1.39)**	**<0.01**	492	577	1.08 (0.88-1.33)	0.45	1.15 (0.91-1.44)	0.24	703	649	**1.42 (1.18-1.71)**	**<0.01**	**1.34 (1.12-1.62)**	**<0.01**

Model 1: Adjusted for age, study level (<Primary, Primary, High school, >High school), age at first birth (25-29, <20, 20-24,>29, nulliparous), age at menarche (12-13, <12, >13), previous biopsies (yes/no), family history of breast cancer. Models for the overall population are further adjusted for menopausal status.
Model 2: Further adjusted for BMI (1-yr before diagnosis).

Figure 4.5: Odds ratio (95%CI) for the association between body mass index (BMI), waist to hip ratio (WHR) and breast cancer incidence (overall and by menopausal status).



Lines represent the ORs (thick lines) and 95% CIs (dotted lines) for breast cancer based on restricted cubic splines for log transformed BMI and WHR with knots at the 10^{th} 50^{th} and 90^{th} percentiles of their respective distributions. The reference was set at the 10^{th} percentile of BMI and WHR distributions. Models account for age, study level, recruitment area, age of menarche, age at first birth, menopausal status, existence of previous biopsies and family history of breast cancer. Models for the WHR also account for BMI.

4.2.3 Association between obesity and other related anthropometric factors and prostate cancer risk.

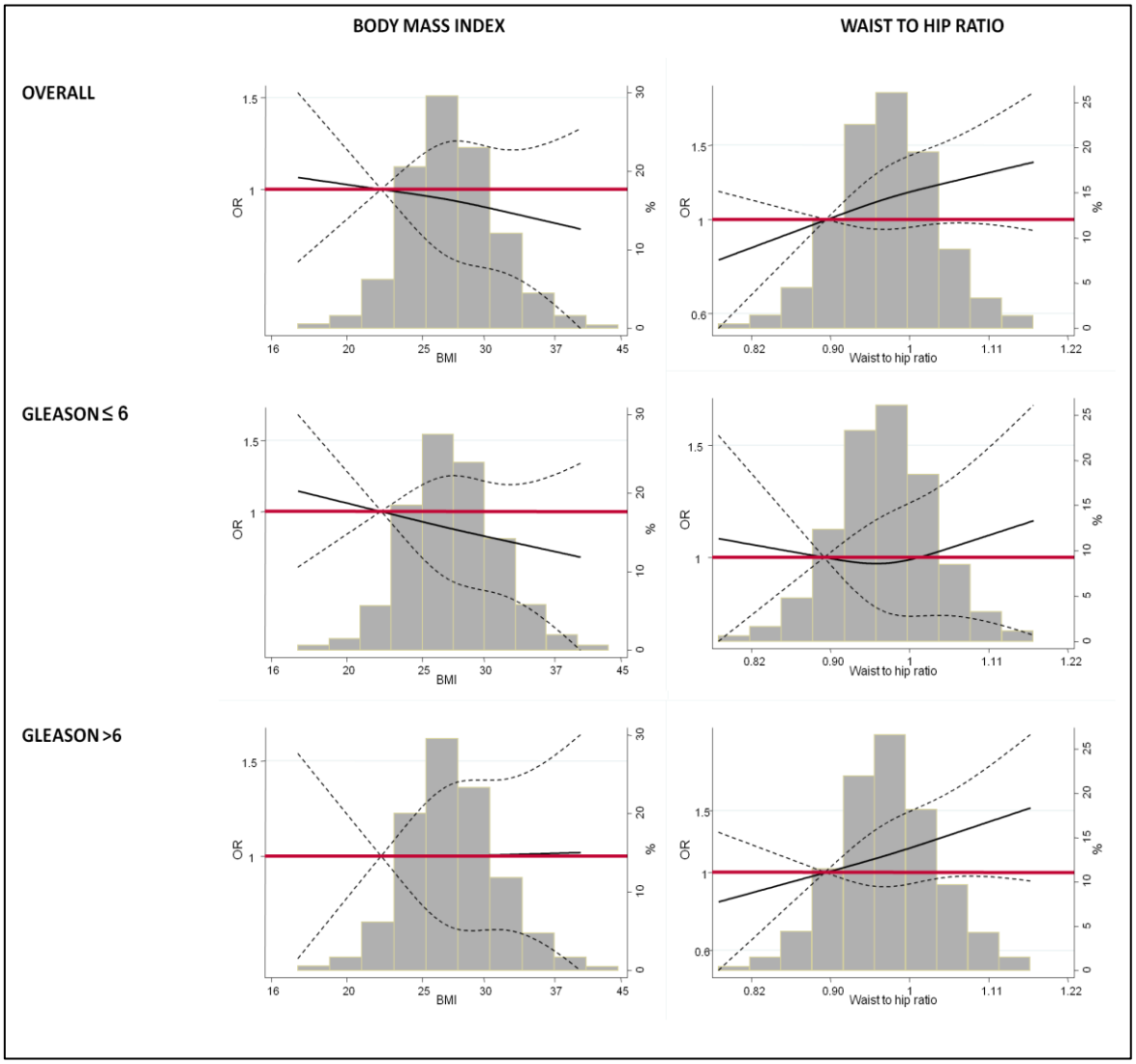
Table 4.10 displays the ORs (95%CI) for the associations between the studied anthropometric variables and prostate cancer risk, overall and by Gleason score at diagnosis. The WHR was the only variable significantly associated with an overall increased risk of this tumor. When analyses were stratified by Gleason score, OR tended to be higher for cancers with Gleason scores >6 ($OR_{0.1\ units}$: 1.21; 95%CI: 1.03-1.43), but no statistically significant differences between low and high grade tumors were observed. However, age at maximum weight was associated with an increased risk of high-grade prostate cancers ($OR_{5\ years}$: 1.05; 95%CI: 1.00-1.10; p-heterogeneity=0.09). On the contrary, an almost significant inverse association of BMI with low-grade tumors was found ($OR_{2\ units}$: 0.94; 95%CI: 0.88-1.01), while the OR for high-grade tumors went on the opposite direction and was not statistically significant ($OR_{2\ units}$: 1.00; 95%CI: 0.94-1.07). Similar to what is shown for breast cancer, figure 4.6 presents the dose-response association for BMI and the WHR, in this case overall and by Gleason score at diagnosis. Again results were very similar when excluding interviews classified as “fair”, when adjusting for self-reported diabetic status or when stratifying the analyses by BMI (data not shown).

Table 4.10: MCC-Spain: Odds ratios (95%CI) for the association between the studied anthropometric variables and prostate cancer: overall and by Gleason Score at diagnosis

	N		Model 1		Model 2		Gleason ≤6			Gleason > 6			
	Controls	**Cases**	**OR (95%CI)**	**p-trend**	**OR (95%CI)**	**p-trend**	**N**	**OR (95%CI)**	**p-trend**	**N**	**OR (95%CI)**	**p-trend**	**p-het**
Age maximum height (per year)	852	770	0.98 (0.94-1.05)	0.52	0.98 (0.94-1.02)	0.52	307	0.97 (0.93-1.05)	0.62	351	0.99 (0.94-1.05)	0.75	0.86
Weight at age 20 (per 5 kg)	1158	920	1.01 (0.94-1.06)	0.72	1.01 (0.96-1.07)	0.60	355	1.02 (0.95-1.09)	0.65	387	1.00 (0.93-1.07)	0.95	0.64
Weight at age 45* (per 5 kg)	1129	955	0.97 (0.95-1.01)	0.16	0.97 (0.93-1.02)	0.26	368	0.96 (0.90-1.02)	0.17	405	0.98 (0.92-1.04)	0.53	0.47
Age maximum weight (per 5 years)	1178	977	1.02 (0.98-1.05)	0.40	1.03 (0.99-1.07)	0.20	387	1.00 (0.95-1.05)	0.97	411	**1.05 (1.00-1.10)**	**0.05**	0.09
Weight gain since age 20 (per 5 kg)	1055	920	0.97 (0.93-1.01)	0.19	0.98 (0.92-1.04)	0.49	355	0.97 (0.89-1.05)	0.42	387	1.00 (0.92-1.07)	0.87	0.56
BMI													
20-24	328	254	1.00		-		103	1.00		102	1.00		
25-29	691	529	0.93 (0.74-1.17)		-		214	0.93 (0.70-1.25)		230	1.02 (0.78-1.37)		
>29	308	235	0.88 (0.67-1.15)	0.34	-		84	0.77 (0.54-1.09)	0.15	98	0.92 (0.65-1.30)	0.65	
Per 2 units BMI	1327	1018	0.98 (0.93-1.02)	0.40	-		401	0.94 (0.88-1.01)	0.08	430	1.00 (0.94-1.07)	0.92	0.10
Waist tertiles													
<81.4 cm	424	300	1.00		1.00		127	1.00		114	1.00		
81.4-93.0 cm	442	316	1.01 (0.80-1.28)		1.03 (0.80-1.32)		136	1.13 (0.82-1.57)		133	1.16 (0.84-1.61)		
≥93.0 cm	447	295	0.96 (0.75-1.22)	0.74	1.00 (0.73-1.39)	0.97	88	0.78 (0.50-1.21)	0.34	144	1.31 (0.87-1.97)	0.18	
Per 10 cm waist	1313	911	0.98 (0.90-1.07)	0.67	1.00 (0.87-1.14)	0.97	351	0.96 (0.80-1.15)	0.68	391	1.12 (0.94-1.33)	0.21	0.15
WHR tertiles													
<0.81	436	248	1.00		**1.00**		108	1.00		89	**1.00**		
0.81-0.88	435	302	1.14 (0.90-1.46)		**1.18 (0.92-1.51)**		130	1.32 (0.96-1.83)		131	**1.47 (1.05-2.04)**		
≥0.88	441	356	1.25 (0.98-1.60)	0.07	**1.32 (1.02-1.72)**	**0.04**	111	1.15 (0.81-1.63)	0.46	168	**1.83 (1.30-2.58)**	**<0.01**	
Per 10 units WHR	1312	906	1.11 (0.98-1.25)	0.11	1.13 (0.99-1.30)	0.08	349	1.18 (0.99-1.40)	0.06	388	**1.21 (1.03-1.43)**	**0.02**	0.69

Model 1: Adjusted for age, study level (<Primary, Primary, High school, >High school), recruitment area, family history of prostate cancer.
Model 2: Further adjusted for BMI (1-yr before interview)
p-het: p for heterogeneity of effects as estimated in model 2

Figure 4.6: Odds ratios (95%CI) for the association between body mass index (BMI), waist to hip ratio (WHR) and prostate cancer incidence, overall and by Gleason score.



Lines represent the ORs (thick lines) and 95% CIs (dotted lines) for prostate cancer based on restricted cubic splines for log transformed BMI and WHR with knots at the 10th 50th and 90th percentiles of their respective distributions. The reference was set at the 10th percentile of BMI and WHR distributions. Models account for age, study level, recruitment area, and family history of prostate cancer. Models for the WHR also account for BMI.

4.3 Results for objective 3

4.3.1 Characteristics of the sample

Table 4.11 shows the main characteristics of the studied participants. Compared to their controls, breast cancer cases were slightly younger and had greater BMI values, while prostate cancer cases presented lower education levels. Among controls, PSA testing was more frequent in diabetic men. Diabetic women were older, had lower education levels and higher prevalence of obesity than their counterparts, while diabetic men presented lower education levels than non-diabetics. Interestingly, diabetic women were more likely to be diagnosed with triple negative tumors than those without diabetes.

4.3.2 Association between diabetes, diabetes duration, diabetes treatment and breast cancer risk

Table 4.12 presents the results for the associations between diabetes status, diabetes management, duration of diabetes and breast cancer risk, overall and by intrinsic subtypes. After multivariate adjustment for age, study level, BMI, age at menarche, age at first birth, existence of previous biopsies and family history of breast cancer, diabetic women showed no overall increased risk of breast cancer (OR: 1.09; 95%CI: 0.82-1.45). However, significant heterogeneity of the effect was observed by intrinsic subtypes ($p_{heterogeneity}$=0.04), with a positive association being encountered for triple negative tumors (OR: 2.13; 95%CI: 1.25-3.63). Diabetic women under conservative management, as well as those under treatment with oral hypoglycemic agents, showed no overall increased risk of cancer, although again the results suggested a heterogeneous effect by tumor subtype. Conservative management was associated with a non-significant risk of developing HER2 tumors, while treatment with oral hypoglycemic agents alone showed a positive link with TN breast cancer. Diabetes treatment with insulin (with or without an oral hypoglycemic agent) was associated with an overall increased risk of breast cancer (OR: 2.14; 95%CI: 1.12-4.09), and this effect was similar across tumor subtypes. Moreover, when analyses were restricted to women under insulin treatment, a non-significant positive dose response association was observed between years of insulin use and breast cancer risk (OR: 1.10; 95%CI: 0.98-

1.23). Interaction analyses revealed no effect modification of BMI on the association between diabetes or diabetes management and breast cancer risk (data not shown).

Analyses based on metformin use alone showed a heterogeneous effect of this drug over different tumor subtypes: while no effect was observed for HER2 or TN tumors, a reduced risk for HR+/HER2- breast cancer was seen with increasing years under metformin use ($OR_{per\ year}$: 0.89; 95%CI: 0.80-0.99). Duration of treatment with sulfonylurea was associated with a non-significant increased risk of breast cancer ($OR_{per\ year}$: 1.05; 95%CI: 0.98-1.13), after adjustment for insulin and metformin use. Although we could not evaluate the dose-response association between years of insulin glargine use and cancer risk due to a lack of power (N=11 women received this type of insulin), we found that women who had ever received insulin glargine (yes/no) had an increased risk of breast cancer when compared to women who had never received this drug before (OR: 4.97; 95%CI: 1.09-22.7).

To conclude with the results based on breast cancer, we evaluated the association between diabetes duration and breast cancer risk. No linear relationship was seen, although results suggested a potential u-shaped dose-response (see figure 4.7).

Table 4.11: MCC-Spain: Main characteristics of the studied MCC-Spain population by diabetic and cancer status

		Breast cancer							Prostate cancer					
		CASES			CONTROLS				CASES			CONTROLS		
		No DM (N=835)	DM (N=81)	p-val$_1$	No DM (N=995)	DM (N=99)	p-val$_1$	p-val$_2$	No DM (N=916)	DM (N=138)	p-val$_1$	No DM (N=1109)	DM (N=232)	p-val$_1$	p-val$_2$
Age		62.0 (8.7)	68.9 (8.0)	<0.01	64.1 (9.14)	71.7 (8.4)	<0.01	<0.01	65.8 (7.3)	67.2 (7.1)	0.05	66.0 (8.5)	69.3 (7.0)	<0.01	0.06
Study level	No studies	168 (20.1)	33 (40.7)		192 (19.3)	41 (41.4)			198 (81.2)	46 (18.9)		204 (18.4)	55 (23.7)		
	<HS	319 (38.2)	27 (33.3)		357 (35.9)	32 (32.3)			355 (87.2)	52 (12.8)		366 (33.0)	74 (31.9)		
	HS	235 (28.1)	15 (18.6)		283 (28.4)	16 (16.2)			209 (88.9)	26 (11.1)		313 (28.2)	57 (24.6)		
	>HS	113 (13.5)	6 (7.4)	<0.01	163 (16.4)	10 (10.1)	<0.01	0.32	154 (91.7)	14 (8.3)	<0.01	226 (20.4)	46 (19.8)	0.28	<0.01
BMI	<25	316 (37.8)	14 (17.3)		462 (46.4)	19 (19.2)			242 (26.4)	19 (13.8)		291 (26.2)	41 (17.7)		
	25-30	338 (40.5)	29 (35.8)		339 (34.1)	38 (38.4)			487 (53.2)	67 (48.5)		581 (52.4)	117 (50.4)		
	>30	181 (21.7)	38 (46.9)	<0.01	194 (19.5)	42 (42.4)	<0.01	<0.01	187 (30.4)	52 (37.7)	<0.01	237 (21.4)	74 (31.9)	<0.01	0.10
Age menarche	<12	171 (20.5)	13 (16.1)		213 (21.4)	23 (23.2)			-	-		-	-		
	12-13	378 (45.2)	39 (48.1)		445 (44.7)	33 (33.4)			-	-		-	-		
	>13	286 (34.3)	29 (35.8)	0.69	337 (33.9)	43 (43.4)	0.09	0.17	-	-		-	-		
Age first birth		27.0 (26.3)	27.0 (4.8)	0.21	26.0 (26.4)	27 (26.4)	0.97	0.75	-	-		-	-		
Family history studied cancer	No	721 (86.4)	72 (88.9)		905 (91.0)	83 (83.8)			749 (81.8)	128 (92.8)		1043 (94.0)	217 (93.5)		
	Yes	114 (13.7)	9 (11.1)	0.52	90 (9.1)	16 (16.2)	0.02	<0.01	167 (18.2)	10 (7.3)	<0.01	66 (6.0)	15 (6.5)	0.77	<0.01
Previous biopsies	No	742 (88.9)	76 (93.8)		979 (98.4)	98 (99.0)			-	-		-	-		
	Yes	93 (11.1)	5 (6.2)	0.17	16 (1.6)	1 (1.0)	0.65	<0.01	-	-		-	-		
Screening behaviours last 5 years															
PSA testing/	No				91 (8.8)	17(15.9)						302 (29.8)	43 (21.1)		
Mamogram	Yes				949 (91.3)	90 (84.1)	0.02	<0.01				711 (70.2)	161 (78.9)	0.02	<0.01
DM characteristics															
Duration of disease (yrs)		-	8.73 (7.9)		-	8.6 (7.1)		0.95		7.5 (8.6)			8.0 (10.0)		<0.01
Treatment	Conservative	-	14 (17.3)		-	21 (21.2)				12 (0.7)			30 (12.9)		
	Drugs	-	67 (82.7)		-	78 (78.8)		0.51		126 (91.3)			202 (87.1)		0.21
Tumor characteristics															
Bilateral	No	798 (97.6)	79 (97.5)		-	-			-	-		-	-		
	Yes	20 (2.4)	2 (2.5)	0.99	-	-			-	-		-	-		
Histologic subtype	Ductal	608 (85.0)	64 (87.7)		-	-			-	-		-	-		
	Lobular	53 (7.4)	4 (5.5)		-	-			-	-		-	-		
	Other	54 (7.6)	5 (6.9)	0.80	-	-			-	-		-	-		
Intrinsic subtypes	RH+, HER2-	529 (73.1)	51 (66.2)		-	-			-	-		-	-		
	HER2+	121 (16.7)	9 (11.7)		-	-			-	-		-	-		
	Triple -	74 (10.2)	17 (22.1)	0.01	-	-			-	-		-	-		
Gleason	<6								368 (49.1)	43 (36.8)					
	≥6								381 (50.9)	74 (63.2)	0.41				

Data in the table are percentages for categorical variables or means ± SD for continuous variables.
p-val1: p-value from chi-square or anova test for differences in the distribution of the studied variables by diabetic status.
p-val2: p-value from chi-square or anova test for differences in the distribution of the studied variables by case/control status.
Numbers in tables may differ because not all tumors could be classified according to intrinsic subtype (breast) or Gleason score (prostate).

Table 4. 12: MCC-Spain: Odds ratios (95CI%) for the association between diabetes, diabetes management and breast cancer, overall and by tumor subtype.

	Overall			HR+/HER2-		HER2+		TN		
	Cases N	**Control N**	**OR (95CI)**	**Cases N**	**OR (95CI)**	**Cases N**	**OR (95CI)**	**Cases N**	**OR (95CI)**	**p-het**
OVERALL POPULATION										
Diabetes										
Non-diabetic	835	995	1.00	529	1.00	121	1.00	74	1.00	
Diabetic	81	99	1.09 (0.82-1.45)	51	1.10 (0.81-1.48)	9	0.91 (0.51-1.60)	17	**2.13 (1.25-3.63)**	**0.04**
Diabetes management										
Non-diabetic	835	995	1.00	529	1.00	121	1.00	74	1.00	
Diabetic, conservative management	14	21	1.10 (0.62-1.95)	6	0.89 (0.35-2.02)	5	**2.00 (0.90-4.45)**	1	*	**<0.01**
Diabetic, treatment with oral hypoglycemic agents	47	70	0.89 (0.63-1.28)	32	0.94 (0.65-1.35)	2	*	11	**2.08 (1.13-3.84)**	**<0.01**
Diabetic, treatment with insulin (+/- oral hypoglycemic agents)	20	8	**2.14 (1.12-4.09)**	13	**2.44 (1.12-5.29)**	2	*	**5**	**4.81 (1.31-17.63)**	0.37
DIABETIC POPULATION										
Metformin use (years)**	35	43	0.94 (0.86-1.03)	24	**0.89 (0.80-0.99)**	3	1.11 (0.95-1.29)	8	1.02 (0.92-1.13)	**0.01**
Sulfonylurea use (years)**	18	24	1.05 (0.98-1.13)	13	1.03 (0.93-1.13)	1	*	4	**1.10 (1.00-1.20)**	0.40
Insulin use (years)**	20	6	1.10 (0.98-1.23)	13	1.11 (0.93-1.33)	2	*	5	1.07 (0.83-1.38)	**0.91**
Time since diagnosis of diabetes (years)	81	99	1.02 (0.97-1.07)	51	1.01 (0.95-1.06)	9	1.05 (0.94-1.17)	**17**	1.03 (0.96-1.10)	0.67

Numbers in tables may differ due to lack of information on tumor receptors in some participants.
*Values are not presented due to the small number of cases in these subgroups.**Based on participants who reported duration of treatment
Models for diabetes and diabetes management are adjusted for age, study level (no studies/<high school/high school/>high school), BMI (continuous), age at menarche, age at first birth, existence of previous biopsies and family history of the studied cancer (none/ one first-degree/ more than one first-degree/second-degree).
Models for metformin and sulfonylureafurther adjusted for insulin treatment (yes/no) and for treatment with other hypoglycemic agent (yes/no), models for insulin are adjusted for treatment with metformn (yes/no) or sulfonylurea (yes/no) while models for time since diagnosis of diabetes further adjusted for diabetes treatment regimen (conservative/oral hypoglycemic agents/insulin or both).

4.3.3 Association between diabetes, diabetes duration, diabetes treatment and prostate cancer risk.

Results for prostate cancer are presented in table 4.13. After multivariate adjustment for age, study level, BMI and family history of prostate cancer, an overall decreased risk of this tumor was observed (OR: 0.74; 95%CI: 0.58-0.94). By Gleason score, the association was mainly confined to low-grade tumors (OR: 0.70; 95%CI: 0.46-0.90. $p_{heterogeneity}$=0.06). Moreover, when effect modification by BMI was explored, only normoweight (OR: 0.64; 95%CI: 0.48-0.87), and not overweight/obese diabetic participants (OR: 0.98; 95%CI: 0.64-1.48) showed this protective effect for low-grade prostate tumors (p for interaction=0.02; data not shown in tables).

Overall, diabetic men under conservative management, as well as those treated with hypoglycemic agents alone, showed a decreased risk of prostate cancer, while this protective effect in those under insulin treatment was only observed against less aggressive tumors (OR: 0.48; 95% CI: 0.25-0.93). Analyses stratified by BMI revealed that the protective effect of hypoglycemic agents use was only observed among diabetic men who were normoweight [$OR_{normoweight}$ (95%CI): 0.66 (0.46-0.93); $OR_{overweight\ or\ obese}$ (95%CI): 1.01 (0.63-1.61); p interaction=0.04]. Results based on the diabetic population only, found no association between years of metformin (OR: 0.97; 95% CI: 0.92-1.02) sulfonylurea (OR: 0.96; 95%CI: 0.93-1.05) or insulin use (1.01; 95%CI: 0.97-1.09) and the risk of prostate cancer. Similarly, no association was observed with insulin glargine treatment (yes/no; N=8 men had ever received this insulin) (OR: 1.64; 95%CI: 0.54-5.00).

Regarding diabetes duration, an almost significant inverse association was observed for prostate cancer (OR: 0.98; 95% CI: 0.94-1.00), and when modeling the dose-response relationship using restricted cubic splines no significant departures from linearity were observed (figure 4.7). The results of sensitivity analyses were similar to those from the main analyses, and we observed no effect modification by "screening habits" (data not shown in tables).

Table 4.13: MCC-Spain: Odds ratios (95%CI) for the association between diabetes, diabetes management and prostate cancer in MCC-Spain, overall and by Gleason score.

	Overall			Gleason ≤6		Gleason >6		
	Cases N	Controls N	OR (95CI)	Cases N	OR (95CI)	Cases N	OR (95CI)	p-het
OVERALL POPULATION								
Diabetes								
Non-diabetic	916	1109	1.00	368	1.00	381	1.00	
Diabetic	138	232	0.74 (0.58-0.94)	43	0.70 (0.46-0.90)	74	0.92 (0.73-1.14)	**0.06**
Diabetes management								
Non-diabetic	916	1109	1.00	368	1.00	381	1.00	
Diabetic, conservative management	12	30	**0.47 (0.23-0.95)**	2	*	10	0.94 (0.43-2.05)	*
Diabetic, treatment with oral hypoglycemic agents	97	160	**0.76 (0.58-1.00)**	35	0.77 (0.54-1.11)	48	0.87 (0.66-1.15)	0.51
Diabetic, treatment with insulin (+/- oral hypoglycemic agents)	29	42	0.84 (0.51-1.38)	6	**0.48 (0.25-0.93)**	16	1.05 (0.66-1.66)	**0.08**
DIABETIC POPULATION								
Duration of metformin use**	79	118	0.97 (0.92-1.02)	26	1.00 (0.92-1.09)	38	0.96 (0.87-1.05)	0.38
Sulfonylurea use (years)**	35	58	0.96 (0.93-1.05)	10	0.92 (0.83-1.04)	16	0.99 (0.88-1.06)	0.44
Insulin use (years)**	24	37	1.01 (0.97-1.09)	5	1.00 (0.91-1.13)	13	1.01 (0.95-1.08)	0.99
Time since diagnosis of diabetes	138	232	0.98 (0.94-1.00)	43	0.94 (0.87-1.00)	74	0.97 (0.92-1.01)	0.38

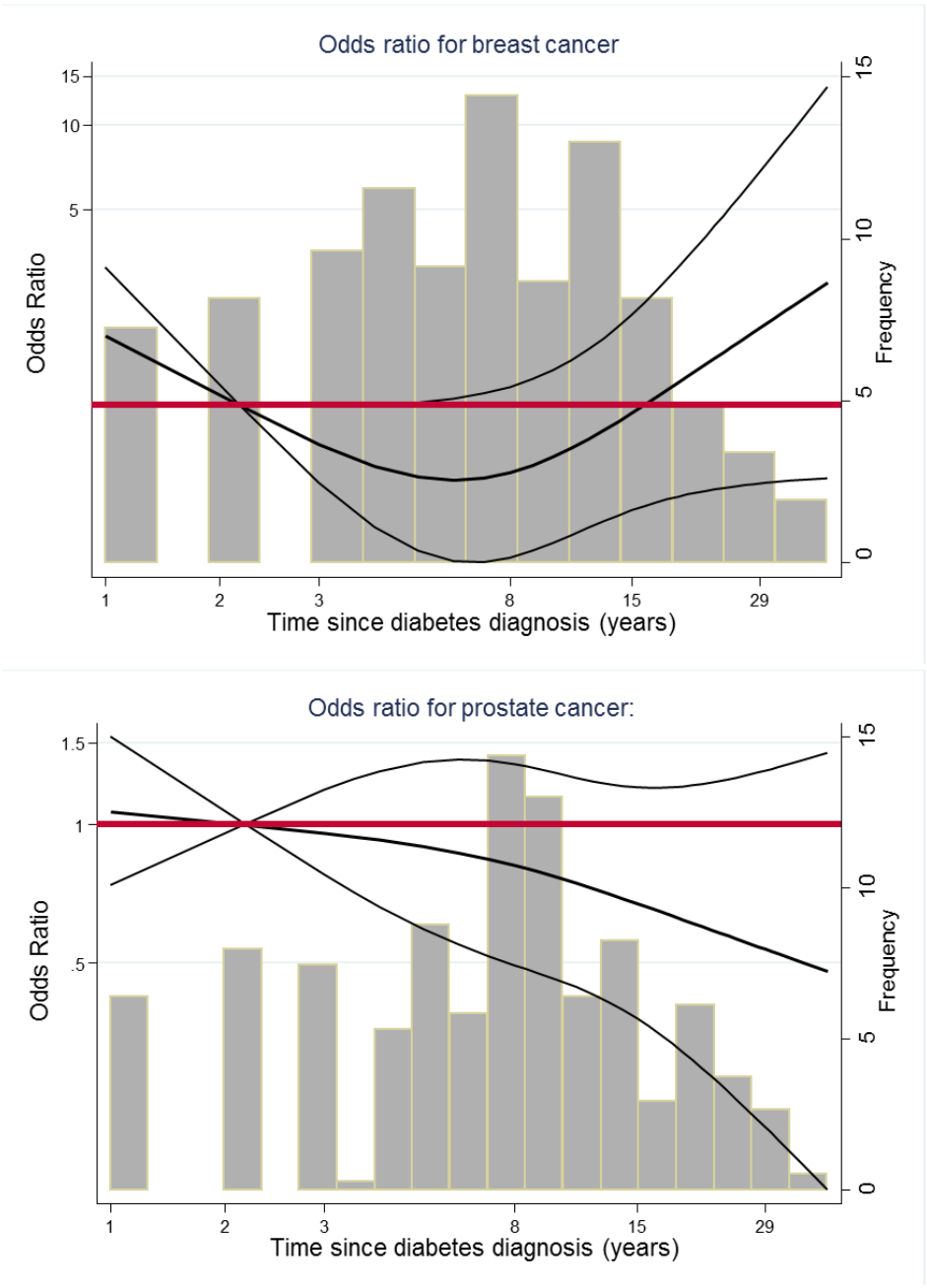
Numbers may differ due to lack of information on Gleason scores in some participants

*Values are not presented due to the small number of cases in this group. **Based on participants who reported duration of treatment

Models for diabetes and diabetes management are adjusted for age, study level (no studies/<high school/high school/>high school), BMI (continuous) and family history of prostate cancer (none/ one first-degree/ more than one first-degree/second-degree).

Models for metformin and sulfonylureafurther adjusted for insulin treatment (yes/no) and for treatment with other hypoglycemic agent (yes/no), while models for time since diagnosis of diabetes further adjusted for diabetes treatment regimen (conservative/oral hypoglycemic agents/insulin or both).

Figure 4.7: Odds ratios (95%CI) for the association between diabetes duration and risk of breast and prostate cancers



Odds ratios for "time since diabetes diagnosis" and cancer risk. Lines represent the odds ratios and 95% confidence intervals for breast and prostate cancer based on restricted cubic splines for log-transformed "time since diabetes diagnosis" with knots at the 10th (2 years), 50th (7 years) and 90th (12 years) percentiles. The reference is set at the 10th percentile of the distribution. Models are adjusted for age, study level (no studies/<high school/high school/>high school), BMI (continuous), diabetes treatment (conservative; oral hypoglycemic drugs; insulin +/- oral hypoglycemic drugs) and family history of the studied cancer (none/ one first-degree/ more than one first-degree/second-degree). Models for breast cancer further adjust for age at menarche, age at first birth and existence of previous biopsies.

5 DISCUSSION

5.1 Discussion related to objective one

5.1.1 Arsenic and cancer mortality

In the Strong Heart Study, a US-based prospective cohort study, low to moderate inorganic arsenic exposure, as measured in urine, was associated with increased mortality from lung, prostate and pancreatic cancers over almost 20 years of follow-up. The associations persisted after adjustment for sociodemographic and behavioral cancer risk factors. Unexpectedly, arsenic exposure was associated with decreased mortality from lymphatic and hematopoietic cancers. Arsenic was not associated with kidney cancer, and for liver cancer the increased risk was small and statistically non-significant. Both tumors have been associated with high arsenic exposure in other populations (145;146). Overall, increasing urinary arsenic concentrations showed a positive but non-significant association with total cancer mortality. Our results extend the associations of arsenic with lung and prostate cancer, observed previously only at high levels of exposure (145;147-149). In addition, we found supportive evidence for pancreatic cancer, a cancer with limited epidemiological evidence available.

Several studies have evaluated the association between lung cancer and high levels of arsenic exposure. In 10,591 participants from Southwestern Taiwan (148), the hazard ratios for lung cancer incidence were 1.09, 2.28, 3.03 and 3.29 for arsenic concentrations in drinking water of 10 to 99, 100 to 299, 30 to 699, and 700 µg/L or more, respectively, compared to less than 10 µg/L. In a case-control study from Antofagasia (Chile), the odds ratio for lung cancer comparing the highest (200-400 µg/L) to the lowest (<10 µg/L) categories of arsenic in drinking water was 8.9 (95%CI: 4.0-196) (150). Lung cancer mortality was also increased among young adults in northern Chile who were exposed to high doses of arsenic in utero (151).

Regarding prostate cancer, ecologic evidence from Southwestern Taiwan, where the population was exposed to high levels of arsenic in drinking water from artesian wells, showed increased prostate cancer mortality compared with the overall Taiwanese population (38). Based on the Taiwanese evidence, the IARC concluded there was evidence for a dose-response relationship (37). In a population from Utah exposed to

moderate arsenic concentrations in drinking water, the SMR for prostate cancer compared to the overall US population was 1.48 (95%CI: 1.07-1.91) (152).

Epidemiological evidence for an association between arsenic and pancreas cancer is scarce. Japanese individuals exposed during infancy to high arsenic levels through contaminated milk showed an SMR for pancreatic cancer of 1.79 (95%CI: 1.23-2.61) compared with unexposed (145). In a case-control study from Spain (153), at low-moderate arsenic in drinking water, the odds ratios for exocrine pancreatic cancer comparing the highest (≥0.11 μg/g) to lowest (≤0.05 μg/g) toenail arsenic quartiles was 2.02 (1.08-3.78).

Few studies have evaluated the association between arsenic and overall cancer mortality. In a prospective cohort including 115,903 participants in Bangladesh, the hazard ratios (95%CI) for cancer mortality were 1.10 (0.77-1.59), 1.44 (1.06-1.95), 1.75 (1.28-2.40) and 1.56 (1.06-2.30) comparing arsenic concentrations in drinking water of 10 to 49, 50 to 149, 150 to 299, and 300 or more to less than 10 μg/L, respectively (154).

In occupational settings (155;156), and in infants exposed through milk powder (145), arsenic exposure has been associated with increased blood cancer mortality. Arsenic trioxide is used in the treatment of some leukemias such as promyelocytic leukemia (157), and is under investigation for multiple myeloma (158). Arsenic trioxide pharmacotherapy has been related to cytotoxicity and apoptosis in cancer cells (159). As we could not specifically evaluate the association between arsenic and specific lymphatic or hematopoietic cancers due to the small number of cases, this finding requires replication in other populations and needs to be interpreted with caution.

Evidence at low-moderate levels remains limited for most cancer. Bladder cancer has been the most frequently studied cancer at these levels of exposure, with inconsistent findings (44;152;160). For lung cancer, a case-control study conducted in New Hampshire and Vermont counties found an odds ratio for small-cell and squamous-cell carcinoma of 2.75 (95% CI 1.39-5.91) comparing toenail arsenic concentrations ≥0.114 vs. <0.05 μg/g. In our study, histological information was not available, and we cannot evaluate if the association found with lung cancer mortality was also related to those cancer types. Finally, at very low levels of exposure, a Danish

cohort of 57,053 participants found no association between arsenic in drinking water (mean 1.2 μg/L, range 0.05 to 25.3 μg/L) and lung, liver, bladder, kidney or prostate cancer incidences over 10 year of follow-up (44). Our study, conducted in a population exposed to low-moderate arsenic levels, extends previous evidence for mortality associated with several cancer types, but included too few cases to evaluate some cancer of interest, including bladder an skin cancers.

Recently, the National Research Council convened a committee to evaluate the health effects of arsenic risk because of controversy regarding the current US EPA arsenic standard in drinking water. This maximum contaminant levels of 10 μg/L was established on the basis of lung and bladder cancer risk in highly exposed populations from southwestern Taiwan, and the risk of arsenic exposure at lower doses was derived using linear low-dose extrapolation. Our findings offer useful information for risk assessment, as provide direct evidence at low-moderate levels and support a linear dose-response relationship for lung, prostate and pancreatic cancers with no evidence of a threshold.

5.1.2 Cadmium and cancer mortality

Low to moderate cadmium exposure, as measured in urine, was associated with increased mortality from overall, smoking-related, lung and pancreas cancer over almost 20 years of follow-up. Our findings are consistent with previous cohort studies showing increased incidence and mortality for overall (72;161), lung (161-163), and pancreas cancers (78;79) in individuals with increased cadmium exposure. Contrary to other studies, however, we found no association with prostate (164-166), breast (74) or kidney cancer (77), although we had limited power to identify associations due to the small number of events.

Smoking, a cause of several cancers including lung and pancreas cancer (167), is an important source of cadmium exposure (49). In our population, the association of cadmium with lung and pancreas cancer remained significant after adjusting for smoking status and pack-years, suggesting that cadmium is an independent risk factors for these tumors, although we cannot discard residual confounding. Moreover, the associations were similar after removing the group of current smokers from the analyses. We also hypothesized that cadmium could act as a mediator of the association

between smoking and lung cancer mortality, and estimated that cadmium explained around 9% of the excess lung cancer mortality due to tobacco smoking. Although this association may seem weak, cadmium is only one of the many carcinogens present in tobacco smoke, and we had one single cadmium measure which could be affected by measurement error.

Women have higher cadmium internal dose compared to men (168), but it is unclear if higher cadmium levels in women are associated with worse health outcomes. In our study, there were no differences in overall or smoking-related cancer mortality by sex, although the association was somewhat stronger in men. In populations from Sweden and the US, urine cadmium has been associated with incident breast (80) and endometrial (169) cancer. In our study we found no association with breast cancer mortality, similar to NHANES III (79), although our results are limited by the small number of breast cancer deaths. We could not evaluate the association between urine cadmium and endometrial cancer mortality as only two women died from this cancer.

Some (76;80;165;166), but not all (170-172) epidemiological studies have found an association between cadmium and prostate cancer. A systematic review showed an increased risk of kidney cancer in cadmium exposed workers (77), but evidence from general populations is lacking. Cadmium has also been proposed as a contributor to liver cancer, with supportive evidence from China (173). Finally, there is some animal evidence that cadmium could induce tumors of the hematopoietic system, although epidemiological evidence is lacking.

In our study, we found no association between urine cadmium and mortality from kidney cancers, and observed a positive but non-significant association with liver and lymphohematopoietic cancer mortality. The small number of deaths in each type of cancers, however, limited our ability to detect associations.

5.1.3 Strengths and limitations

Strengths of this study include the prospective design and long follow-up, the low rate of follow-up loses and the careful standardization and quality control of data collection and laboratory analyses (133;174). Furthermore, this study provides information on cancer mortality in American Indians, and understudied population whose cancer experience and cancer determinants have not been well described. The

relatively high concentrations of urine cadmium and arsenic in this population when compared to the general US population suggest that arsenic and cadmium exposure may be important environmental risk factors for cancer development in American Indians.

Our study has some limitations. First, cancer mortality is an imperfect outcome to study tumors with relatively good prognosis. Second, we relied on death certificates to identify the cause of death and had no confirmation from hospital records or cancer registries. Cancer deaths, however, are considered to be better coded than other causes of death. Third, we could not exclude participants with cancer at baseline. Analyses excluding cancer deaths during the 2 and 5 years of follow-up, however, showed similar results. Fourth, we had limited statistical power for individual cancer locations and for conducting effect modification analyses. Fifth, we could not account for family history of cancer or for clustering of arsenic exposure. Finally, we only had a single spot urine sample. Recent studies have indicated that urine cadmium in populations exposed to low-moderate levels might not reflect chronic cadmium exposure (175), although uncertainties in exposure measurement are likely to result in non-differential measurement error and to underestimate the associations. Additionally, different strategies to account for urine dilution, yielded similar results. In the case of arsenic, previous evidence in the Strong Heart Study showed relatively constant concentrations over a 10-year period (137), indicating that a single measure reflects long-term arsenic exposure in this population.

5.2 Discussion related to objective two

Results from MCC-Spain add strength to previous findings, while provide new evidence on the association between obesity and breast cancer by intrinsic subtypes. Our data support the hypothesis that central adiposity is a key aspect to consider when studying the connection between obesity and hormone-dependent tumors. In fact, even though BMI did not increase the risk of premenopausal breast cancer, central adiposity was positively associated with premenopausal breast cancer in models that took into account both anthropometric characteristics. In the case of PC, only central adiposity was associated with the risk of high-grade tumors. Our results also point out to the existence of differential effects of weight gain on BC risk by menopausal status and tumor subtype, while show that the age at maximum weight is only associated with the risk of high-grade prostate tumors. Finally, results for anthropometric factors earlier in life show that growth speed is inversely associated with the incidence of all BC subtypes, while does not affect PC risk.

5.2.1 Obesity, other related anthropometric characteristics and breast cancer risk

Despite the numerous studies that have evaluated the relationship between obesity and breast cancer, major uncertainties still exist. Overall, the evidence from dose-response meta-analyses suggests a positive association between BMI and breast cancer risk in post- (176;177) but not premenopausal women (178). This opposing effect, which is also observed in MCC-Spain, is attributed to differences in the biosynthesis of estrogens by menopausal status (179). While in premenopausal women the ovaries are the main site of estrogen production and this process is under homeostatic regulation, in postmenopausal women the ovaries are replaced by peripheral site synthesis (180). Additionally, the greater degree of insulin resistance observed in postmenopausal women (181), as well as the increased tendency for young obese women to have anovulatory menstrual cycles (101), could act as contributing factors. In agreement with our findings, studies focusing on the effects of central adiposity on breast cancer risk have shown that while central adiposity is positively associated with the risk of postmenopausal breast cancer independently of BMI (182), this relationship is only observed in premenopausal women after adjustment for overall

adiposity (182-185). Although the exact mechanisms are unknown, these results indicate that differences in metabolic effects between general and central adiposity must exist.

Breast tumors are a very heterogeneous group of diseases with different risk factors, molecular and clinical characteristics. However, few reports have explored the possible role of obesity on specific breast cancer molecular subtypes, and when this has been done it has mostly relied on classifications that only considered estrogen and progesterone receptor status (186-188). A meta-analysis of 31 studies evaluating the association between relative body weight (highest versus reference categories) and ER/PR defined breast cancer showed that the risk for ER+/PR+ tumors decreased among pre- and increased among postmenopausal women (186). In order to allow for comparisons with this meta-analysis we reclassified our tumors according to ER and PR, independently of HER2 status, and found very similar results: Obese premenopausal women showed a decreased risk of ER+/PR+ tumors (OR=0.65; 95%CI: 0.38-1.02) while postmenopausal obese women presented an increased risk (OR=1.79; 95%CI: 1.21-2.13).

Contrary to our results, a meta-analysis on case-case and case-control studies focusing on the association between obesity and triple negative tumors showed that only obese pre-, and not postmenopausal women presented an increased risk of this particular cancer subtype (189). Potential factors that could link obesity to TN tumors have been described elsewhere, and would include insulin-related as well as inflammatory-related mechanisms (189). However, no biological mechanism has been proposed that could explain a differential effect of obesity on the risk of TN tumors by menopausal status. In MCC-Spain, the stronger association between obesity and breast cancer risk was precisely seen in postmenopausal women that developed TN tumors (OR=2.31; 95%CI: 1.30-4.11). A recently published case-control study in Japan, also reports an increased risk of triple negative cancers among obese postmenopausal women (190). Because we only had four cases of TN tumors among obese premenopausal women, we cannot draw any conclusion for this specific group.

Overweight and obesity earlier in life have been consistently associated with a decreased risk of breast cancer (191-193). Although our results do not support an overall effect of "weight at age 20" on breast cancer risk, they suggest that early obesity

could decrease the risk of certain tumor subtypes in premenopausal women. In this sense, women who had a higher weight at age 20 or who reached their maximum weight earlier in life showed a decreased risk of premenopausal HER2+ or TN tumors. Results indicate the exact opposite effects for postmenopausal women, but the significance of this heterogeneity is unknown and should be evaluated in future studies.

Weight gain in adulthood has shown to increase the risk of postmenopausal breast cancer in numerous cohort studies (194-197), with evidence from a recently published meta-analysis that this excess risk could be confined to HR+ tumors (186). In contrast, most published studies on premenopausal women have either reported null findings (198;199) or inverse associations for weight changes and risk of HR+ tumors (188;190). Our results initially suggested a positive association between weight gain since age 20 and overall breast cancer risk in postmenopausal women, but this association was attenuated after accounting for current BMI. Similarly, by tumor subtype, a positive association with the risk of postmenopausal HR+/ERB2- tumors was only observed in models that did not account for BMI. Conversely, a decreased risk of HER2+ breast cancer was seen with increasing weight gain, a finding that to our knowledge has never been reported before.

Our results are in accordance to those of some prospective cohorts showing that the age at maximum height is associated with the risk of developing BC, independently of other pubertal/reproductive events (192;193;200;201). In this sense, earlier adolescent growth spurt would lead to precocious exposure of the immature breast tissue to hormonal and growth factors, at a time of increased susceptibility to carcinogenesis. Additionally, exposure to these factors during adolescence could influence the maturation of the hypothalamic pituitary ovarian axis, which regulates ovarian hormone production (202).

5.2.2 Obesity, other related anthropometric characteristics and prostate cancer risk

In epidemiological studies, obesity is positively associated with prostate cancer mortality (203), while it displays a dual effect on prostate cancer incidence, with an increased risk for advanced cancers and a decreased risk for non-aggressive tumors (111). Our results support a link between obesity and the risk of high-grade tumors, although only the WHR showed a statistical significant association. It has been suggested that the dual effect of obesity on the risk of low- and high-grade tumors could be due to the effect of low testosterone levels in obese men, as this hormone would lose the capacity of controlling the differentiation of prostate cells with the consequent growth of more aggressive tumors (204). Obesity, particularly central obesity, is also associated with increased serum levels of estradiol, insulin, insulin like-growth factors or cytokines, all of which have been related to an increased risk of aggressive prostate tumors (205). Additionally, obese men are less likely to have an early diagnosis due to their increased plasma volume with lower PSA levels, and their excess prostate tissue growth and fat deposition (206).

The effect of weight gain during adult life on the risk of prostate cancer has been less explored, and results from published studies are inconsistent, with some showing a positive association (207;208), while others do not (208;209). Results from the Multiethnic Cohort, including more than 83,000 men, showed that self-reported weight changes since age 21 were associated with an increased risk of prostate cancer in Whites and African Americans, but not in Japanese men (210). In Europe, results from the Nord-Trøndelag Health Study with 20,991 adult men followed-up for 9 years, showed no differences in prostate cancer risk with changes in measured weight (209). Similarly, in the Melbourne Collaborative Cohort Study, that investigates 17,045 men, weight gain was not associated with an increased risk of prostate cancer, although in this last study a deleterious effect of weight gain on prostate cancer mortality was observed (208). Our results failed to show a link between weight gain since age 20 and the incidence of this tumor. However, they support an association with age at maximum weight, suggesting that for PC the amount of weight gained may not be as important as the moment during adulthood when this occurs.

5.2.3 Strengths and limitations

Since the present results are based on a case-control study, several methodological limitations exist. First, self-reported data are subjected to recall bias. However, misclassification would be non-differential, particularly for exposures occurring during adolescence (age maximum height) or early adulthood (weight at age 20, age at maximum weight). Also, by calculating BMI before the occurrence of the disease we are reducing the probability that the exposure is affected by the disease. In addition, not all variables are self-reported, and measures of central adiposity were obtained using standardized protocols. Second, the sample size was limited to test differences per tumors subtype, and this limitation is particularly relevant when performing stratified analyses by menopausal status. This stratification, however, is justified taking into account the opposite effects of obesity in pre-and postmenopausal women. In this sense, the results are interesting but should be considered as exploratory. Strengths of this investigation include the use of histologically confirmed incident cases, as well as the wide geographic variability, with cases recruited in many different Spanish regions. Additionally, we could perform analyses by intrinsic subtypes (breast) or Gleason scores (prostate), which is essential to correctly address the epidemiology of these tumors. Finally, our results are robust to various sensitivity analyses.

5.3 Discussion related to objective three

Our findings do not support an overall association between diabetes and breast cancer, although they suggest an increased risk of triple negative tumors in postmenopausal diabetic women. Women treated with insulin showed a greater incidence of HR+ and triple negative subtypes, while metformin use seemed to reduce the risk of HR+ breast cancer. Finally, treatment with hypoglycemic agents, and in particular sulfonylureas, was associated with an increased risk of TN tumors.

Regarding prostate cancer, diabetes showed a protective effect on the incidence of low-grade tumors, particularly in men who were normoweight and in those who were not taking insulin. The protective effect of diabetes was stronger as time since diagnosis increased.

5.3.1 Diabetes and breast cancer risk

Despite a number of meta-analyses linking diabetes and breast cancer in postmenopausal women (211;212), results from three recently published large population based studies have raised uncertainty. The first of these null studies, retrospectively evaluated the risk of breast cancer in postmenopausal women using data from the Columbia Linked Health Database, which covers around 4.5 million residents (213). The second, based on register linkage of the Danish National Diabetes register and Cancer Registry, and representing the whole Danish population, found no association between diabetes prevalence or diabetes duration and breast cancer risk (214). The third, which included 68,019 postmenopausal women followed over a mean of 11.8 years, also failed to find an overall increased risk of breast cancer among those with diabetes (215). However, results from this last study were modified when diabetes medication was taken into account, as a reflection of the importance of considering diabetes treatment when studying the risk of cancer associated to this disease. In this sense, women under treatment with "drugs other that metformin" showed a non significant increased risk of breast cancer (HR: 1.16; 95%CI: 0.93-1.45), while those receiving metformin presented lower incidence (HR: 0.75; 95%CI: 0.57-0.99).

Some authors have suggested that TN tumors may be more strongly associated with insulin resistance (216). Our results, showing a strong association between diabetes and incidence of TN tumors, would support this hypothesis. However, the few previous

epidemiological studies that have evaluated the influence of diabetes on breast cancer by molecular subtypes, have yielded contradictory results. In the Carolina Breast Cancer Study, a population-based case-control study carried out in 24 counties in central and eastern North Carolina, an increased risk of basal-like breast tumors was seen in pre- and postmenopausal women with higher waist circumference and waist to hip ratio (217). Although these measures of central adiposity are well known markers of insulin resistance and in fact were strongly associated with a history of diabetes (217), no elevated prevalence of this metabolic disease was found in women with triple negative tumors when compared to other breast cancer subtypes. Results from the Study of Osteoporotic Fractures, including 8,956 women with different components of the metabolic syndrome showed that diabetes was associated with a borderline significant increased risk of ER+ and PR+ cancers, while no effect was seen for ER- or PR- (218). Finally, a retrospective cohort study focusing on the metabolic syndrome as a whole, instead of diabetes as an individual component, found a higher prevalence of this syndrome in patients with TN tumors, with blood glucose being an independent risk factor of this specific subtype (219). In light of these conflicting results, additional research is warranted.

The biological mechanisms behind a potential increased risk of breast cancer in women with diabetes are unknown, although are probably related to alterations in circulating concentrations of insulin, insulin-like growth factors and endogenous sex hormones. Insulin and IGF-1 receptors are frequently expressed in breast cancer cells (219-221), with evidence that their signaling pathways are of crucial importance in the role of breast cancer tumorigenesis (222;223), particularly in the case of TN tumors (224). Insulin also has a paracrine effect on secretion of adipokines (225;226), which may contribute to the increasing risk of more aggressive breast tumors (227). Additionally, insulin can inhibit the production of sex hormone-binding globulin in the liver (228), with subsequent increased levels of free estradiol and increasing proliferation of breast epithelial cells.

5.3.2 Diabetes and prostate cancer risk

A reduced incidence of prostate cancer has been previously reported in diabetic men (126;229), with substantial evidence showing that the magnitude of this inverse association becomes higher with increasing diabetes duration (128;214;230-234).

Although some studies have found that diabetes may increase the risk of more aggressive prostate tumors (235), results from a recently published meta-analysis suggest that men with diabetes are protected for both low (RR:0.74; 95%CI: 0.64-0.86) and high-grade (RR:0.78; 95% CI 0.67-0.90) disease (236). We failed to find a protective effect of diabetes on the risk of high-grade tumors, even though our diabetic population showed a higher prevalence of PSA testing that that observed in non diabetics. One possible explanation is the higher prevalence of obesity among diabetic men, as obesity has been associated with elevated incidence of aggressive prostate tumors (205;237).

Several mechanisms for decreased prostate cancer incidence among diabetic men have been proposed, including the adoption of healthier lifestyles after diabetes diagnosis. The stronger inverse association with long-standing diabetes has been explained by beta cell exhaustion leading to insulin depletion and reductions in IGF-1 (125;230;238), as these two factors have potent growth-stimulatory effects on the prostate (239;240). Hyperglycemia is also thought to play a major role in protecting against prostate cancer development (241), with epidemiological evidence showing that strict glycemic control increases the risk of this tumor (242). Some authors have also hypothesized that decreased levels of testosterone, a known stimulatory agent of prostate growth, may protect diabetic men from prostate cancer development. Still, it remains unclear if reductions in circulating testosterone are correlated with decreased intra-prostatic levels of this hormone (238). Finally, genetic studies have shown that individuals with greater susceptibility to type 2 diabetes may have a decrease risk of prostate cancer (243-246).

5.3.3 Diabetes treatment and risk of hormone-dependent tumors

Metformin is the most commonly used drug in patients with type 2 diabetes (129). Experimental studies have shown that this biguanide is capable to inhibit the proliferation of breast (247;248) and prostate cancer cells (249). Additionally, it can induce apoptosis of triple negative (250) and HER2 positive cells (251), and it can represses the process of epithelial to mesenchymal transition (252). Interestingly, metformin also reduces the growth of several tumoral xenografts in mice including those established from breast and prostate cancer cells (249).

The evidence on the association between metformin use and the risk of breast cancer has been mixed in epidemiologic studies (253-257). Results from most meta-analyses have suggested a non significant decreased risk of breast cancer in metformin users (253;255-257), while no effect has emerged from Randomized Clinical Trials (253;258). Only one previous study, based on data from the Women´s Health Initiative clinical trials, has evaluated the incidence of specific subtypes of breast cancer in diabetic women under metformin treatment (215). Interestingly, findings from this study were very similar to those from MCC-Spain, with a protective effect of metformin only observed for ER+/ PR+ and not ER-/PR- tumors.

Regarding prostate cancer, the evidence is highly consistent, and most observational studies and Randomized Clinical Trials have shown no effect of metformin use on the risk of this tumor (253). Consistently with our findings, data from a recently published retrospective cohort of 119,315 Canadian men, this lack of association is common to low and high-grade cancers (259).

A small number of observational studies have shown an increased risk of several cancers (260-262) and overall cancer mortality (263) in diabetic patients treated with sulfonylurea, when compared to other treatments. However, results from Randomized Clinical Trials have failed to demonstrate a significant effect on the risk of overall cancer (258). The evidence for breast cancer and prostate cancer is scarce and the few existing studies have yielded inconsistent results (264-268). Our results point towards a possible detrimental effect of sulfonylureas on the risk of breast cancer.

Insulin is a well-known growth factor with particularly strong mitogenic effects over cancer cells (269). Results from a meta-analysis of observational studies has lately shown an overall increased risk of cancer in patients treated with insulin, although no significant increased risk of breast (RR:1.86; 95%CI:0.92-2.98) or prostate cancers (RR:1.17; 95%CI:0.92-1.49) was reported (270). Similarly, a recently published cohort study over 498,407 Taiwanese fails to encounter any association between insulin use and prostate cancer risk (271). Data from Randomized Controlled Trials (RCTs) do not support the hypothesis that insulin therapy increases the risk of cancer, although interpretation of their results is limited because cancer has not been the end point of interest. Additionally, the few RCTs on insulin therapy that have reported data on cancer have focused on mortality and not incidence (272).

Available data from in vitro experiments suggests that insulin glargine may have greater proliferative effects than human insulin in some breast cancer cell lines (272;273). This finding is supported by observational studies showing that glargine use may be associated with an increased risk of breast cancer (274-276), at least at high doses and with long duration of treatments (128;272). A population based cohort with more than 27,000 users of insulin glargine and 100,757 users of NPH has shown a 30% increased risk of breast cancer in patients with ever use of glargine (RR:1.3: 95%CI: 1.0-1.8) (277). Some experimental studies also suggest that glargine may have a potent mitogenic activity on prostate cancer cells (278), but the evidence from epidemiological studies is less consistent, with some studies showing an increased risk (276;279), while others have not (275;277;280).

5.3.4 Strengths and limitations

This is the first population-based study that evaluates the association between diabetes and cancer in the Spanish population. Similarly to what it was explained in objective 2, our main strength is that we have histologically confirmed incident cases and that we have been able to classify them according to their receptor status (breast) or Gleason scores (prostate). Very few observational studies have previously evaluated the association between diabetes and breast cancer subtypes, and our results suggest the importance of this approach.

This study also has several limitations. First, diabetes history is self-reported and so it is subject to recall bias. According to the International Diabetes Federation, around 35% of the European diabetic population is unaware of having this condition (281), and similar rates have been described in Spain (282). However, we expect under-diagnosis not to be so important in our population because participants had frequent contact with the health system, as reflected by their high prevalence of screening practices. Additionally, results from a meta-analysis on the association between diabetes and breast cancer showed that findings were unchanged when the diagnosis of diabetes was self-reported or confirmed with medical records (212). Second, we were limited by the sample size, particularly for evaluating certain subgroup associations. Third, our dataset does not allow us to differentiate between type 1 or type 2 diabetes, although by excluding all cases diagnosed of diabetes before the age of 45 we are limiting the

probability of including cases of type 1 diabetes. Fourth, it is hard to disentangle the effects of diabetes treatment from those of the disease itself. As an example, diabetic patients receiving insulin are a subgroup with very specific characteristics, they usually have more severe forms of diabetes and greater prevalence of comorbidities that can lead themselves to an increased cancer risk. Additionally, individuals receiving insulin usually visit their doctors more frequently, and this may increase their probability of screening and cancer detection. Finally, we do not have information on glycaemic control, and this has been shown to be an important independent predictor of cancer risk (283).

6 CONCLUSIONS

Objective 1:

Conclusion 1: Low to moderate exposure to inorganic arsenic was prospectively associated with increased mortality from lung, prostate and pancreas tumors, and decreased mortality from cancers of the lymphatic and hematopoietic tissue

Conclusion 2: Low to moderate exposure to cadmium was prospectively associated with increased mortality from overall cancer, smoking-related tumors and lung and pancreatic cancer.

Conclusion 3: The relatively high concentrations of urine cadmium and arsenic in the studied communities, when compared to the general US population, suggest that arsenic and cadmium exposure may be important environmental risk factors for cancer development in American Indians.

Objective 2:

Conclusion 1: In postmenopausal women, obesity and central adiposity were associated with an increased risk of breast cancer, and this association was similar for all tumor subtypes. The age at maximum height was associated with a decreased risk of all tumor subtypes, while the age at maximum weight was inversely associated only with the risk of triple negative tumors. Finally, differential effects of weight gain were observed by tumor subtype.

Conclusion 2: In premenopausal women, central adiposity was associated with an increased risk of breast cancer in models that accounted for body mass index, while obesity early in life was mainly associated with a decreased risk of more aggressive tumor subtypes.

Conclusion 4: In men, the age at maximum weight and the waist to hip ratio were positively associated with the risk of high-grade prostate tumors.

Objective 3:

Conclusion 1: In postmenopausal women, previous diagnosis of type 2 diabetes was associated with an increased risk of triple negative breast tumors.

Conclusion 2: Insulin use was associated with an overall increased risk of breast cancer. Metformin use was associated with a decreased risk of HR+/HER2- tumors, while use of sulfonylurea was associated with an increased risk of TN cancers.

Conclusion 3: For prostate cancer, a decreased risk of low-grade tumors was observed in normoweight men with diabetes, and this protective effect was stronger as time since diagnosis increased.

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ANNEXES

CURRICULUM VITAE
Esther Garcia Garcia-Esquinas, MD, MPH.

Updated October 2nd

PERSONAL DATA

Name:	García García-Esquinas, Esther	**Telephone:**	0034-676-37-37-53
Nationality:	Spanish	**E-mail:**	esthergge@gmail.com
Date and place of Birth:	Oct 26, 1981 Madrid (Spain)	**Address:**	Avenida Paralela Nº 17 2ºA . Urb. Las Mimbreras Bloque 8. 28220 Madrid (Spain).

CURRENT POSITION

Research personnel contracted by the Carlos III Health Institute ("Río Hortega" contract, co-funded by the European Regional Development Fund).

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Advisor: Marina Pollán (mpollan@isciii.es)

EDUCATION

2009-2012	**Diploma of Advanced Studies. Doctoral program in Public Health.** Autonomous University of Madrid, Spain.
2007- 2010	**Medicine Residency in Preventive Medicine and Public Health*.** Ramón y Cajal Hospital, Madrid *4-year specialized degree program accredited by the Spanish Ministry of Health and Education
2007-2009	**Graduate Diploma in Design and Statistics in Health Science** Autonomous University of Barcelona
2006 -2007	**Master of Public Health** National School of Public Health. Carlos III Health Institute. Madrid (Spain)
1999 -2005	**Degree in Medicine and Surgery*. (MD.)** **School of Medicine** **Autonomous University of Madrid, Madrid, Spain** *6-year Spanish professional degree in Medicine and Surgery.

PROFESSIONAL EXPERIENCE

2011 –2013	**"Río Hortega" health research competitive contract (**National Center for Epidemiology**).** This included a six-month training fellowship at the Division of Occupational and Environmental Health Unit at John Hopkins Bloomberg school of Public Health.
2010	**Research personnel contracted by CIBERESP (Ciber for Epidemiology and Public Health)**
2007- 2010	**Residency in Preventive Medicine and Public Health** Hospital Universitario Ramón y Cajal, Madrid (Spain).

COMPETITIVE RESEARCH PROJECTS

Project title: Role of metals in the genesis of prostate cancer in Spain: metal-MCC-Spain. PI12/00150

Duration: 2013-2015

Budget: 188155 €.

Principal Investigator: Beatriz Pérez-Gómez

Project title: Epidemiologic Study of night shift work, circadian disruption, genetic susceptibility and breast and prostate cancer risk. PI11/01889

Duration: 2012-2014

Budget: 181091 €.

Principal Investigator: Manolis Kogevinas

Project title: Etiopathogeny of gastric Cancer in Spain: Possible interactions microbiota-environment in a population-based multicenter case-control study (MCC-Spain Gastric Cancer). PI11/01403

Duration: 2012-2014

Budget: 210320 €.

Principal Investigator: Nuria Aragonés Sanz.

TEACHING EXPERIENCE

Academic course 2010-2011	**Master in Public Health (National School of Health)** Epidemiology and Biostatistics courses.
Academic course 2010-2011	**International Master in Public Health (National School of Health)** Epidemiology and Biostatistics courses **Master in Public Health and Field *Epidemiology Training Program* (National School of Health)**Outbreak investigations in Public Health.
Academic course 2009-2010	**Master Program in Nursing, European University (Alcala de Henares, Madrid)** SPSS in Medical Science Clinical Trials: evidence based medicine.
2007	"Food Manipulation Course". Instituto Madrileño de Administración Pública (IMAP) Hospital Ramón y Cajal. Madrid, November 2007 (20 h).

RESEARCH CENTER NETWORKS

Biomedical Research Centre Network for Epidemiology and Public Health (CIBEResp)

GRANTS FOR ACADEMIC STUDIES ABROAD

2010	**European Educational Program in Epidemiology (EEPE). Florence. Italy.** Grant funded by the Spanish Society of Epidemiology.
2009	**John Hopkins University. Bloomberg School of Public Health** (Department of Environmental Health Science). Grant funded by the Spanish Ministry of Health
2000-2001	**Erasmus program. Facoltá di Medicina e Chirurgia Universitá Degli Studi di Parma. Italy.** (1 academic year)

INVESTIGATION AWARDS

*****CiberESP award for the Thesis Project "Metal Exposures, Endocrine Factors and Cancer Risk". Encuentro para la Excelencia de la Investigación en Salud Pública 2013. Lazareto de Maó, Menorca

*****7th Conference on Metal Toxicity and Carcinogenesis 2012: "Best poster" award.

***** Enrique Nájera award for the research project entitled "Metalohormones and cancer". National School of Public Health 2011**.** 6.000 euros award to help young epidemiologists to implement a research project.

Spanish Society of Epidemiology Award for Young Epidemiologist – 1st place for the project entitled "Gastric Cancer mortality trends in Spain: Regional differences and disease burden due to premature death, 1975-2005". Gerona 2008. XXVI Reunión Científica de la Sociedad Española de Epidemiología.

Spanish Society of Epidemiology Award for Young Epidemiologist – 1st place for the project entitled "Heavy metals in breast milk and its relationship with sociodemographic variables, lifestyle factors and diet: BioMadrid proyect". Córdoba, Octubre 2007. XXV Reunión Científica de la Sociedad Española de Epidemiología.

First place ACCESIT award. X for the project entitled "Research study on Medical records: documentation and proper vaccine administration in splenectomized at the Severo Ochoa Hospital" XVI Jornadas de Vacunación Internacional sobre Actualización de Vacunas. Hospital Universitario 12 de octubre.

*****AWARDS RELATED TO THIS DOCTORAL THESIS

** **García-Esquinas E,** Pérez-Gómez B, Lope Carvajal V, Castaño G, Altizabar JM, Merino Salas S, Capelo R, Guinó E, Pollán M. Type 2 diabetes and hormone-dependent tumours: MCC-SPAIN. Sociedad Española de Epidemiología. Granada. 2013

García-Esquinas E, Jiménez-Moleón JJ, Linares C, Aragonés M, Kogevinas M, Molina AJ, Guevara M, Moreno V, Pollán M. Type 2 diabetes and digestive tumours: MCC-SPAIN. Sociedad Española de Epidemiología. Granada. 2013

Linares C., **García-Esquinas E**, Castaño G, Fernández T, Guevara M, Llorca J, Peiró R, Fernández-Somoano A, Aragonés N. Anthropometric factors and gastroesophageal cancer: MCC-SPAIN. Sociedad Española de Epidemiología. Granada. 2013

García-Esquinas E, Loeffler L, Weaver V, Fadrowski J, Navas-Acién A. Kidney function and tobacco smoke exposure in US adolescents. International Society for Environmental Epidemiology. Basel, 2013.

** **García-Esquinas E,** Pollán M , Umans JG, Francesconi KA, Goessler W, Guallar E, Farley J, Yeh J, Best LG, Navas-Acien A. Arsenic exposure and cancer mortality in a US-based Prospective Cohort Study: the Strong Heart Study. International Society for Environmental Epidemiology. Basel, 2013.

Fernández-Navarro P, García-Pérez J., González-Sánchez M., **García-Esquinas E.**, López-Abente G., Astray J., Fernández M.A., Martínez M., Aragón N.. Association between blood mercury levels and proximity to industrial facilities. Environmental Health 2013. Boston, 2013.

** **García-Esquinas E,** Pollán M , Umans JG, Francesconi KA, Goessler W, Guallar E, Farley J, Yeh J, Best LG, Navas-Acien A. Arsenic exposure and cancer mortality in a US-based Prospective Cohort Study: the Strong Heart Study. 7th conference on Metal Toxicity and Carcinogenesis. Alburquerque, 2012

** **García-Esquinas E,** Tellez-Plaza M, Pollán M , Umans JG, Francesconi KA, Goessler W, Guallar E, Farley J, Yeh J, Best LG, Navas-Acien A. Cadmium exposure and cancer mortality in a US-based Prospective Cohort Study: the Strong Heart Study. 7th conference on Metal Toxicity and Carcinogenesis. Alburquerque, 2012

** **García-Esquinas E,** Lope V, Pérez- Gómez B, Castaño-Vinyals G, Gómez-Acebo I, Altzibar J, Ardanaz J,Martín V, Tardón A, Alguacil J, Crous-Bou M, Peiró R, Jiménez-Moleón JJ, Pollán M. Fat distribution and adult weight gain as risk contributors in hormone-dependent tumors: MCC-Spain. International Society for Environmental Epidemiology. South Carolina, 2012.

Lope V, **García-Esquinas E**, Aragonés N, Kogevinas M, Dierssen-Sotos T ,Altzibar J, Guevara M, Martin V, Tardón A, Alguacil J, Crous-Bou M, Salas D, Jiménez-Moleón JJ, Pollán M. Birth and childhood characteristics and risk of adult hormone-dependent cancers: MCC-Spain. International Society for Environmental Epidemiology. South Carolina, 2012.

Benavente Y, Cassabonne D, Pérez-Gómez B, García-Esquinas E, Moreno V, Souto A, de Sanjosé S, Martín V. Antecedentes familiares de cáncer en los progenitores de los controles del proyecto MCC-Spain: resultados preliminares. XXV Reunión SEE, Santander, 2012.

Seoane-Mato D, **García-Esquinas E**, Pérez-Gómez B, Romero B, Del Campo R, Aragonés N. Systematic revisión of the prevalence of Helicobacter Pylori in Spain. XXIX Reunión SEE-SESPAS, Madrid. 2011.

García-Esquinas E, Pérez-Gómez B, López V, Aragonés N, Boldo E, Sierra A, Tabernero A, Burgos J, Kogevinas M, Pollán M. Prostate cancer and its association with birth factors: MCC-Spain. XXIX Reunión SEE-SESPAS, Madrid. 2011.

Garcia-Esquinas E, Apostolou A, Fadrowski J, McClain P, Weaver V., Navas-Acien A.Secondhand tobacco smoke: a source of lead exposure in US children and adolescents. Oral communication. International Society for Environmental Epidemiology. Barcelona, 2010.

García-Esquinas E, Aragonés N, Fernández MA, Pérez-Gómez B et al. Mercury, lead and cadmium in human milk in relation to diet, lifestyle and socio-demographic factors in Madrid, Spain. International Society for Environmental Epidemiology. Dublin, 2009.

MJ Perez_Elias, Gutierrez C, Casado J, Muriel A, **Garcia-Esquinas E.** et al, Agreement Degree between two genotype interpretation Systems, Tipanavir (TPV), and Darunavir (DRV) Validated Weight Scores (WS) and Standford HIVdv Program. 5th IAS conference on HIV Pathogenesis, Treatment and Prevention,Cape Town, 2009.

García-Esquinas E. "Gastric Cancer mortality trends in Spain: Regional differences and disease burden due to premature death, 1975-2005". XXVI Reunión Científica de la Sociedad Española de Epidemiología. Gerona, 2008.

Paniagua-A, Varela C, Aragon C, **García-Esquinas E**., Moya JL, Ruiz S, Lahera M. Estudio Caso-Control de La función valvular cardíaca en pacientes tratados con dopaminérgicos ergóticos o similares con hiperprolactinemia. 50 Congreso Nacional de Endocrinología y Nutrición SEEN, Bilbao 2008.

García-Esquinas E., Robustillo Rodela A., Díaz-Agero C., Saa Requejo C. et al. Pacientes colonizados por Staphilococo Meticilin Resistente: ¿Cuándo levantar el aislamiento? Experiencia del Hospital Ramón y Cajal. IX Jornadas Nacionales Sobre Avances en Medicina Preventiva. Murcia 2008. España.

García-Esquinas E, Zuza I, Martínez B. Estudio sobre la documentación y adecuación de las vacunaciones en pacientes esplenectomizados en el Hospital Severo Ochoa. Oral communication. IX Jornadas Internacionales Sobre Actualización en Vacunas. Hospital Universitario 12 de Octubre. Madrid, 2008.

*****COMMUNICATIONS RELATED TO THIS DOCTORAL THESIS***

García-Esquinas E, Pérez-Gómez B, Fernández-Navarro P, Fernández MA, de Paz C, Pérez-Meixeira A, Gil E, Iriso A, SanzJC, Astray J, Cisneros M, de Santos A, Asensio A, García-Sagredo JM, Frutos García J, Vioque J, López-Abente G, Pollán M, González MJ, Martínez M, Aragonés N. Lead, mercury and cadmium in umbilical cord blood and its association with maternal epidemiological variables and birth factors. BMC Public Health. 2013 Sep 12;13(1):841.

***García-Esquinas E,** Pollan M, Umans JG, Francesconi KA, Goessler W, Guallar E, Howard B, Farley J, Yeh J, Best LG, Navas-Acien A. Arsenic Exposure and Cancer Mortality in a US-based Prospective Cohort: the Strong Heart Study. Cancer Epidemiol Biomarkers Prev. 2013 Jun 25.

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García-Esquinas E, Loeffler LF, Weaver VM, Fadrowski JJ, Navas-Acien A. Kidney function and tobacco smoke exposure in US adolescentes. Pediatrics. 2013 May;131(5).

García-Esquinas E, Pérez-Gómez B. Tobacco and breast cancer risk. GeySalus (Journal for the Spanish Breast Cancer Research Group GEICAM) 2013

García-Esquinas E*, Apostolou A*, Fadrowski JJ, McClaine P, Weaver VM, Navas-Acien A. Secondhand Tobacco Smoke: A source of Lead Exposure in US Children and Adolescents. Am J Public Health. 2011. * These authors contributed equally to this project.

García-Esquinas E, Pérez-Gómez B, Fernández MA et al. Mercury, lead and cadmium in human milk in relation to diet, lifestyle habits and sociodemographic variables in Madrid (Spain). Chemosphere 2011 Sep; 85(2) :268-76

García-Esquinas E, López-Gay D. Paludismo en la Comunidad de Madrid años 2004 - 2008.

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García-Esquinas E, Pérez-Gómez B, Pollán M, Boldo E. Gastric cancer mortality trends in Spain, 1976-2005, differences by autonomous region and sex. BMC Cancer. 2009 Sep 28;9:346.

García-Esquinas E, Zuza Santacilia I, Martínez Mondéjar B. Documentation and proper vaccine administration in splenctomized patients. Med Clin (Barc). 2009 Apr 8.

García-Esquinas E, Gènova Maleras R, Esteban Vasallo MD, Domínguez Berjón MF. Objetivo 3. Iniciar la vida en buena salud. Informe del Estado de Salud de la Población de la Comunidad de Madrid 2009. Dirección General de Atención Primaria. Consejería de Sanidad, Comunidad de Madrid, 2009:99-112.

García-Esquinas E., Aragonés N, Fernández MA, Pérez-Gómez B, González MJ, Iriso A, Astray J, Pollán M, Martínez M. Mercury, Lead and Cadmium in Human Milk in Relation to Diet, Lifestyle and Socio-Demographic Factors in Madrid, Spain. Epidemiology. 2009 Nov 20;6: p S151.

García-Esquinas E, Aragonés N, Fernández M, Astray J, Pérez-Gómez B, Martínez M, García JF, Gil E, González MJ et al. Metales pesados en leche materna y su relación con variables socio-demográficas, hábitos y dieta: Proyecto Bio-Madrid. Gac Sanit.21:55, 2007.

Manuscripts accepted for publication:

***García-Esquinas E**, Pollan M, Téllez-Plaza M, Francesconi K, Goessler W, Guallar E, Umans JG, Yeh J, Best L, Navas-Acién A.Cadmium Exposure and Cancer Mortality in a Prospective Cohort: the Strong Heart Study. Accepted for publication on Environmental Health Perspectives, June 2013 (Ref 13-06587)

Manuscripts under review:

García-Esquinas E, Fernández-Navarro P, Pérez-Gómez B, Fernández MA, de Paz C, Pérez-Meixeira A, Gil E, Iriso A, SanzJC, Astray J, Cisneros M, de Santos A, Asensio A, García-Sagredo JM, Frutos García J, Vioque J, López-Abente G, Pollán M, González MJ, Martínez M, Aragonés N. Newborns and low to moderate prenatal environmental lead exposure: might fathers be the key?. Sent on July 2013 to Environmental Research.

Manuscripts already drafted as a first author waiting for the MCC-Spain Steering Committee approval:
***Associations of diabetes and diabetes treatment with breast and prostate cancer incidence by histological subtype: a case-control study (MCC-Spain)

***Obesity, fat distributio, weight changes in adulthodd and risk of hormone-dependent tumors in a multicenter case-control study (MCC-Spain).

*** *MANUSCRIPTS RELATED TO THIS DOCTORAL THESIS*

CONTINUOUS EDUCATION AS A GRADUATE STUDENT

2012	**Epigenetics – Biology, Methods, and Biostatistics** Held at the Annual Conference of the International Society for Environmental Epidemiology (ISEE)
2012	**DAGs in Everyday Life: Design, Implementation, and Interpretation** Held at the Annual Conference of the International Society for Environmental Epidemiology (ISEE)
2012	**Immunology of environmental diseases.** Held at the Bloomberg School of Public Health
2012	**Environmental and occupational epidemiology.** Held at the Bloomberg School of Public Health
2011	**Statistical analyses in genetic epidemiology using R** Held at the Center for Research in Environmental Epidemiology (CREAL) Total hours: 20 hours
2011	**Introduction to *Geographic Information Systems*** Held at the Spanish National School of Public Health, Madrid (Spain). Total Hours: 20 h
2011	**Stata Corp's Net Course 151. Introduction to Stata Programming** Six weeks on line course
2010	**Statistical analyses with R** Held at the Spanish National School of Public Health, Madrid (Spain). Total Hours: 30 h.
2010	**Basics of Access** Held at the Spanish National School of Public Health, Madrid (Spain). Total Hours: 20 h.
2009	**Epidemiologic Methods I y Epidemiologic Methods II.** * Held at the Bloomberg School of Public Health
2009	**Statistical Methods In Public Health I y Statistical Methods In Public Health II** Held at the Bloomberg School of Public Health
2008- 2009	**Multivariable Regression Analysis in Health Sciences** Held at the Autonomous University of Barcelona (Spain) Total hours: 325 h.
2009	**Multiple Regression Analysis. Logistic Regression using SPSS** Held at the Ramon y Cajal Hospital, Madrid (Spain) Total hours: 32 h.
2009	**Demography and Health** Held at the National Public Health Institute, Madrid (Spain) Total hours: 45 h.
2008	**Methodological and conceptual advances in Clinical research investigation and EBM** Held at the Lain Entralgo Agency, Madrid (Spain) Total hours: 18 h.
2008	**Survival analysis. Kaplan-Meier survival curves and Cox Regression** Held at the Lain Entralgo Agency, Madrid (Spain) Total hours: 32 h.
2008	**Biomonitoring human exposure to environmental pollutants in Spain** Held at the International University of Andalucía, Sevilla (Spain) Total hours: 30 h.
2008	**Poisson regression** Held at the Spanish National School of Public Health, Madrid (Spain). Total Hours: 30 h.

2008	**Meta-analysis** Held at the Spanish National School of Public Health, Madrid. (Spain). Total Hours: 30 h.
2007-2008	**Fundamental Statistics** Held at the Autonomous University of Barcelona (Spain.) Total hours: 325 h.
2008	**Writing and publishing Scientific Papers** Held at the Spanish National School of Public Health, Madrid. (Spain). Total hours: 30 h.
2008	**System Dynamics Application in Epidemiology** Held at the Spanish National School of Public Health, Madrid (Spain). Total Hours: 30 h.
2008	**Surveillance and Infection Control** Held at the Public Health Institute, Madrid. (Spain) Total hours: 25 h.
2008	**Biostatistical Methods in Epidemiology** Held at the Autonomous University of Madrid. (Spain) Total hours: 30 h.
2007	**Burden of Disease: Methodology and Applications** Held at the Spanish National School of Public Health, Madrid. (Spain). Total hours: 20 h.
2007	**Data Analysis with SPSS** Held at Autonomous University of Barcelona (Spain.) Total Hours: 50 h.
2006	**Course in bibliographic research** Held at the Lain Entralgo Agency, Madrid (Spain) Total hours: 10 h.

COMPUTER SKILLS

Statistical packages	**Stata** (advanced), R, SPSS, Epiinfo.
Operative systems	Microsoft Windows.

LANGUAGE SKILLS

Spanish	Mother tongue.
English	Excellent written and spoken communication skills.
Italian	Excellent written and spoken communication skills.
French	Basic

OTHER

Acupunture Degree by the Complutense University of Madrid.

RESIDENCY ROTATIONS

From March 2012-September 2012	**Fellowship at Johns Hopkins Bloomberg School of Public Health** **Department of Environmental Health Science** Advisor: Ana Navas-Acien.
From March 2010-May 2010	**Applied epidemiology department. National Center for Epidemiology** Changes in mortality during transmission of pandemic influenza Advisor: Fernando Simón Soria.
From January 2010-March 2010	**Transmissible diseases section of the National Public Health Institute.** Study of imported malaria in travelers and immigrants.
From August 2009-Decembre 2009	**Fellowship at Johns Hopkins Bloomberg School of Public Health** **Department of Environmental Health Science** Advisor: Ana Navas-Acien.
From May 2009 to August 2009	**Department of Health. National Public Health Institute. Madrid** Analysis of Infant Mortality Rates in Madrid Autonomous Community Advisor: Ricard Génova Maleras.
From January 2009 to May 2009	**Biostatistics Unit, Ramon y Cajal Hospital.** Consultant Analyst on the International Europreval Project: socio-economic impact of food allergies in Spain Advisor: Victor Abraira.
From August. 2008 to December 2008	**Rotation in Primary Health Care Coordination Area, Area 7 of Madrid Autonomous Community.**
From April.2008 to August. 2008	**Resident-Fellow. National Center of Epidemiology, Carlos III Institute, Madrid (Spain).** Area of Environmental Epidemiology and Cancer. Advisor: Nuria Aragonés.